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Rancidity in Lean Fish Muscle. I. A Proposed Accelerated Copper-Catalyzed Method for Evaluating the Tendency of Fish Muscle to Become Rancid[1]

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ABSTRACT

A method that can be carried out within 24–72 hr is suggested for determining the tendency of fish muscle to become rancid. It consists of adding measured, trace amounts of copper ion to muscle that has been blended with water (1:3) followed by storage at 0°C. Rancidity is observed subjectively by noting the odours that develop and objectively by means of the thiobarbituric acid reaction.

INTRODUCTION

This series of papers will be primarily concerned with the study of oxidative rancidity in the flesh of frozen and unfrozen Atlantic lean fish, particularly cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*).

Until quite recently it has generally been taken for granted that with fillets of cod, haddock, or sole, problems of oxidative rancidity are either non-existent or are of relatively little importance. Nevertheless, the muscle of all lean fish does contain highly unsaturated lipids, which under certain conditions are capable of becoming strongly rancid. Processors of these fish do not consider it worthwhile to add an antioxidant to either fresh or frozen fillets. Most of the scientific studies concerned with the deterioration of frozen lean fish muscle point to lipid hydrolysis and protein denaturation as the principal mechanisms involved (Dyer and Fraser, 1959).

Relatively few studies have been made to determine the conditions that are conducive to the development of oxidative rancidity in lean fish, and it is not known whether their susceptibility to rancidity varies from season to season, or from one geographical area to another. There are two obvious reasons for the very limited amount of work that has been done on lipid oxidation in lean fish. First, there is very little pressure for such work from the industry. At the present time there appears to be little or no recognized monetary loss resulting from deterioration caused by oxidative rancidity in either fresh or frozen lean fish. Second, it is difficult to apply the usual procedures for studying oxidative rancidity to lean fish muscle.

The quantity of lipid material in the muscle is relatively small, about 0.5–1.5%. Instead of being in a free state, the fats (chiefly phospholipids) are intimately associated with the muscle proteins. As a result, one must constantly bear in mind that any factor affecting the proteins may indirectly affect the

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lipids. The muscle lipids contain some highly unsaturated components which add to the difficulty of extracting them without further accelerating oxidation. Many of the analytical procedures used in studying the development of rancidity in oils and fats are of little value when applied to fish muscle. Furthermore, since lean fish are comparatively resistant to oxidation in the frozen state, very long periods of storage are frequently required to produce any observable rancidity. And on the other hand, if the fish are not frozen, the rapid growth of bacteria and other microorganisms blocks lipid oxidation.

The purpose of the work outlined in this paper has been to develop some procedures that can be adapted to the study of rancidity in lean fish.

EXPERIMENTAL PROCEDURES

Preliminary Studies

In some preliminary experiments (Castell *et al.*, 1962) it was observed that trace amounts of iron or copper ion added to whole or blended cod fillets produced a series of odours that ranged from "seaweedy" to that of strongly rancid fish oil. It was found further that the rate of development of these odours was affected by the addition of antioxidants, chelating agents, and various other chemical compounds, and that the results could be observed with storage periods of 18–72 hr at 0°C. Attempts to extract the lipids from these samples showing various degrees of rancidity and then to measure the extent of oxidation were not successful. However, the thiobarbituric acid (TBA) test applied directly to the tissues without preliminary extraction (Tarladgis *et al.*, 1960) gave very promising results that seemed to correlate well with the organoleptic ratings.

Taken together, these observations suggested that a relatively simple method might be developed for studying some of the factors affecting the rate of development of rancidity in lean fish muscle. This method involves adding to the fish muscle a range of copper ion concentrations above and below that required to produce rancidity under a standard set of conditions, and comparing this with a similar set that differs only by the factor under study; the results to be recorded in TBA values as well as in organoleptic ratings. After many trials, the following procedures were adopted for the purpose of further experimentation.

Preparation of the Blended Fish Muscle

Fish muscle homogenates were prepared from fillets carefully cut from well-iced, gutted fish that were not more than 3 days old. The whole fillets were blended for 45 sec with twice their weight of distilled water, using a 1-gal Waring blendor at low speed. This homogenate was distributed in 75-g amounts in 250-ml Erlenmeyer flasks. Twenty-five millilitres of the solution containing the metal ion was added to the homogenate and mixed by a vigorous rotary motion. A separate flask was prepared for each TBA test that was to be made. The flasks were corked and immediately stored at 0°C. By preparing them in this way the homogenates always contained 1 part by weight of fish muscle and 3 parts of added water or solution. The solutions of copper salts were

prepared to give the required parts per million (ppm) of copper ion when added to the homogenate in 25-ml amounts. The recorded concentrations of metal ion always refer to the amount in the homogenate and not to the fish muscle alone.

Fillets were cut from the fish under sanitary conditions and special effort was made to prepare homogenates with an initially low bacterial content. Other than rapid chilling and holding at 0°C, nothing extra was done to retard bacterial growth in the homogenates during storage. In control samples to which metals were not added, spoilage odours resulting from microbial activity usually did not become evident until the storage period had reached 5–7 days at 0°C. The rancid odours, on the other hand, usually became quite pronounced before 24 hr.

The Thiobarbituric Acid Test

There are several variations in the procedures used for applying the TBA test to fatty foods. The method described by Tarladgis *et al.* (1960) has been found the most satisfactory for use with fish muscle homogenates. Briefly it is as follows: Forty grams of the homogenate (containing 10 g of fish muscle) was mixed with distilled water to bring the final volume to 110 ml. This was acidified to pH 1.5–1.6 by adding approximately 2 ml of 4*N* HCl. The acidified mixture is distilled at a rate to give a total of 50 ml of distillate in 10–12 min. Five millilitres of the distillate is combined with 5 ml of 0.02*M* TBA solution in 90% acetic acid, heated in a stoppered flask in boiling water for 35 min, and then cooled for 10 min in running tap water. The optical density of the solution was read in a spectrophotometer at 538 mμ. Blanks, using distilled water, were run at the same time as the samples.

EXPERIMENTAL RESULTS

As organoleptic evaluation is the ultimate criterion of rancidity, it was necessary first to record the odours resulting from the addition of copper ion to the fish muscle homogenate and then to determine how these odours correlated with the measure of rancidity as indicated by the TBA values.

Description of Rancid Odours

No matter how fresh the muscle may be, it always has a certain amount of odour which is usually characteristic of the species. During the early stages of copper-catalyzed rancidity in lean fish muscle there are faint odours suggesting dried or salted fish. This is followed by more pronounced odours suggesting those associated with sea water and the exposed algae on the rocks at low tide. For this reason the term seaweedy has been used. The seaweedy odours then become oily or rancid and finally develop into the unmistakable odour of rancid fish oil. Occasionally in the later stages a distinct paint-like or linseed oil odour is encountered. If 50 ppm Cu^{++} is added to the homogenate this series of odours is generally encountered within 24 hr at 0°C. If smaller concentrations of the metal ion are used the rate of odour development is retarded and the later stages, including the rancid fish oil odours, may never be reached. With

the development of the advanced stages of rancidity there is a tendency for the homogenate to become slightly discoloured to shades of yellow or yellowish brown.

Copper Concentration and Rancid Odours

Homogenates were prepared over a period of 3 winter months from 20 different lots of cod and haddock fillets, and copper chloride was added to give various Cu^{++} concentrations. The results are summarized in Table I. In general, 20 ppm Cu^{++} invariably brings about the development of strong seaweedy or rancid odours in 24 hr; 7–10 ppm also cause seaweedy odours, but to a lesser extent. Five parts per million or less produce little or no off-odours under these same conditions. There is reason to believe, however, that the sensitivity of the muscle to copper-induced rancidity varies with the season, being greater in the winter and early spring and decreasing considerably in the late spring and summer months (this is dealt with in a later paper of this series).

Table I. Development of rancid odours in cod and haddock muscle homogenates containing increasing amounts of Cu++ and stored 24 hr at 0°C.

	Cu++ added (ppm)				
Odours produced	2	5	7	10	20
	%	%	%	%	%
Strong seaweedy rancid	0	0	0	0	75
Seaweedy	0	0	10	40	25
Slightly seaweedy	0	20	30	60	0
No off odours	100	80	60	0	0

TBA Values and Organoleptic Rancidity

Over a period of several months, 187 samples of fish muscle homogenates containing Cu^{++} (1–50 ppm) were graded for odour and the TBA values were determined. The results in Fig. 1 show that the samples can be divided into three groups.

Although there is some overlapping of TBA values in the three odour categories, correlation between the odours and the TBA values was generally good. Control samples of fresh fish muscle to which no metal had been added had TBA values between 0.02 and 0.03. The earlier, mild seaweedy odours usually develop when the TBA values range between 0.05 and 0.20; from values of 0.20 to about 0.50 the seaweedy odours become more intense; the samples with strong oily, rancid, fish oil, and paint oil odours generally have values between 0.50 and 0.70. Values above 0.82 were not encountered in this work.

Effect of Copper Concentration on the Development of Rancidity

Fish muscle homogenate containing 0, 8, 14, 16, and 20 ppm added Cu^{++} was stored for 24 hr at 0°C. At this point TBA determinations were made.

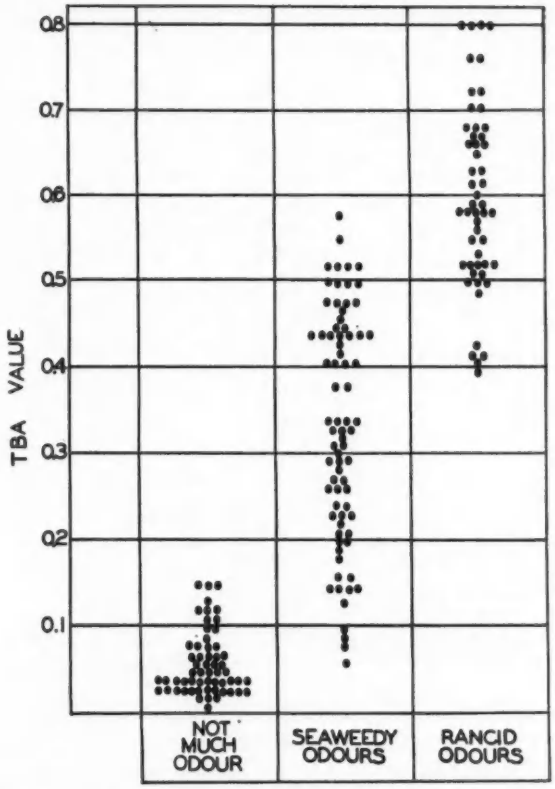


FIG. 1. Relation between organoleptic assessment of rancidity and TBA values in stored cod and haddock muscle homogenates containing Cu++.

In addition to reading the optical densities at 538 mμ a series of other readings was made in the range between 400 and 700 mμ (Fig. 2). The maximum values were found between 520 and 540 mμ. This agrees well with the standard TBA procedure which recommends reading at 538 mμ.

It has been suggested that some metals, and especially copper, may react with the TBA reagent to give coloured compounds. It is possible, therefore, that the results of the tests shown in Fig. 2 may merely indicate the amount of copper present and not the development of oxidative rancidity in the fish muscle. In order to check this hypothesis, tests were made on fish to which 20 ppm Cu^{++} had been added, but which had not been given time to develop any observable rancidity. For comparison, similar samples containing 20 ppm Cu^{++} were stored for 5 days at 0°C, at which time they had developed very strong oily-rancid odours. In addition, there were control samples to which no Cu^{++} had been added. The optical densities at 538 mμ were as follows: rancid fish

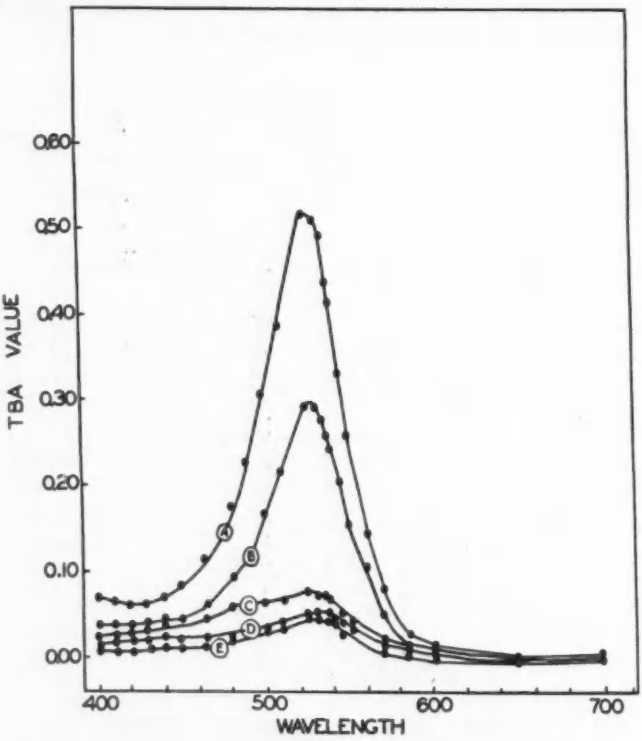


FIG. 2. Effect of wavelength and Cu++ concentration on TBA values of muscle homogenates; (A) 20, (B) 16, (C) 14, (D) 8, (E) 0 ppm Cu++.

muscle containing 20 ppm Cu^{++}, 0.60; non-rancid fish muscle containing 20 ppm Cu^{++}, 0.04; control samples containing no added Cu^{++}, 0.05. These results show that the TBA values of the non-rancid fish which contained copper were essentially the same as the fish without copper. The TBA values on the copper-catalyzed rancid fish, therefore, are not simply a measure of the added copper, but are the result of some compound or compounds formed during the development of rancidity. It was also found that the addition of 50 ppm Cu^{++} to a blank gave no increase in reading over a blank without added copper.

The effect of Cu^{++} concentration on the TBA values for five different lots of muscle from winter-caught fish is shown in Fig. 3. These curves are typical of many more that were obtained in similar experiments. There is a gradual increase in the TBA values with increasing Cu^{++} concentrations up to a point somewhere between 10 and 20 ppm. This is followed by an abrupt rise and then a leveling off. Increasing the concentration of Cu^{++} beyond this point resulted in little or no change in the TBA values. With most fish caught and tested over a 1-year period, this maximum TBA value with increasing Cu^{++}

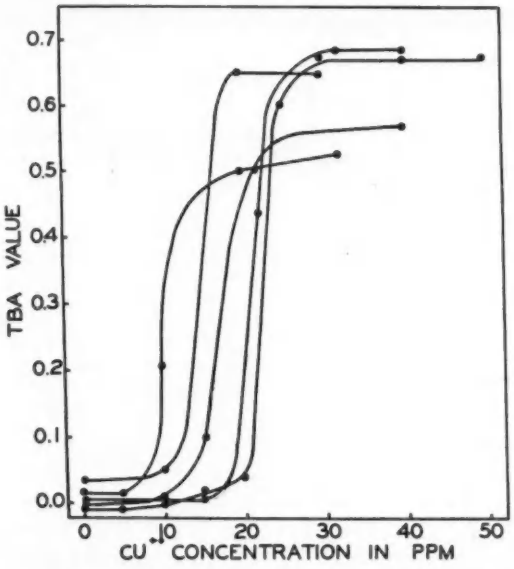


FIG. 3. Effect of Cu++ concentration on the development of rancidity, as indicated by TBA value, after 24 hr at 0°C, for five experiments made with muscle from winter-caught fish.

concentration was between 0.50 and 0.75. Concentrations greater than 30 ppm Cu^{++} will cause a more rapid development of rancidity but not a greater maximum value as judged by TBA or odours.

EFFECT OF STORAGE TIME AT 0°C ON THE DEVELOPMENT OF Cu^{++}-INDUCED RANCIDITY

Three cod muscle homogenates containing 20 ppm Cu^{++} were stored at 0°C and samples were examined periodically for odour and TBA values. The TBA results are given in Fig. 4. During the first 6 hr there was no observable change. Between 18 and 36 hr the TBA curves ascended steeply, followed by plateaus similar to those shown in Fig. 3. Further storage beyond 6 or 7 days is complicated by the growth of psychrophylic bacteria and decomposition of the fish muscle. In spite of this, in another experiment (Fig. 5) there was little or no further change in the TBA values up to 34 days, at which time the muscle had become partially digested and strongly putrid as a result of microbial action. The samples to which 20 and 50 ppm Cu^{++} had been added became strongly rancid within 48 hr and rancid odours persisted for about 10 or 11 days. Following this, the first bacterial odours became evident and subsequently masked the rancidity. It is interesting to note, however, that once they had been formed, the compounds responsible for the TBA reaction persisted even after the muscle had been badly decomposed.

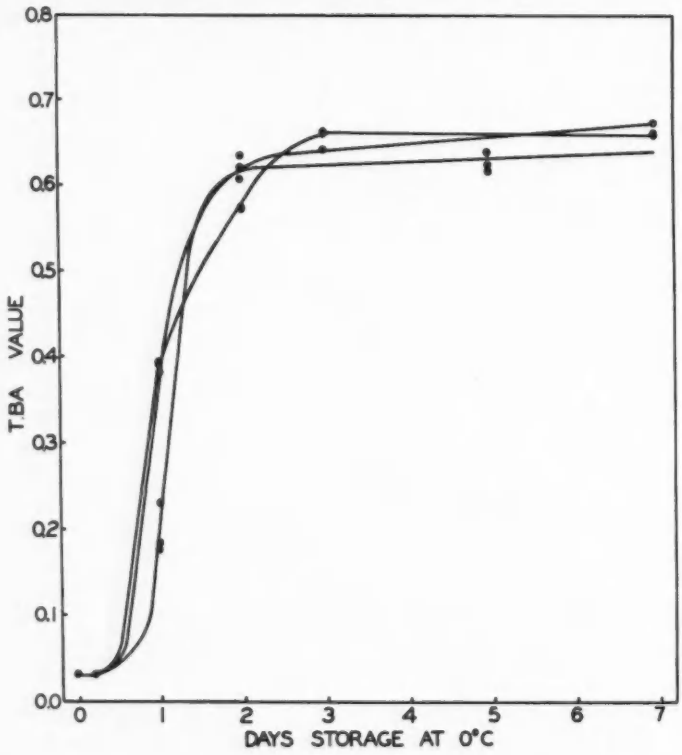


FIG. 4. Relation between storage time and TBA value for cod muscle homogenate containing 20 ppm Cu++ and held at 0°C.

There are two other variations in procedure that are of interest from a theoretical standpoint, as well as being very important in carrying out the proposed test for rancidity. These are considered in the following two sections.

PROPORTIONS OF FISH AND WATER

The procedure used in these investigations calls for 3 parts of water to 1 part of fish muscle in preparing the homogenates. The importance of this ratio to the develpoment of rancidity is shown in Fig. 6. As the proportion of water increases between 1:1 and 1:5 there is a very rapid increase in the development of rancidity. A further dilution of 1:10 made no significant change over that obtained with 1:5. The experiment was repeated a third time and the dilutions were increased as far as 1:20. The horizontal portion of the curve continued as an almost straight line with all the dilutions used that were greater than 1:5.

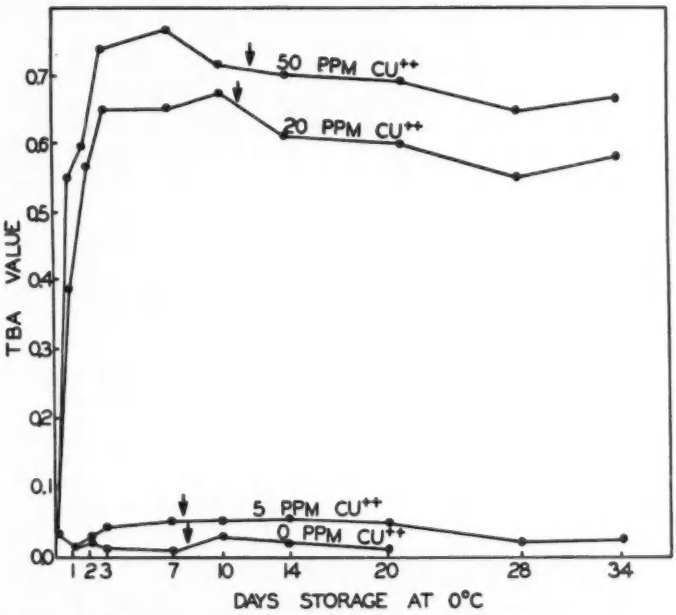


FIG. 5. TBA values of cod muscle homogenates stored at 0°C during a period of 34 days. The samples contained 0, 5, 20, and 50 ppm of added Cu++. The arrows indicate the first evidence of bacterial odours.

ADDITION OF COPPER IONS BEFORE AND AFTER BLENDING

Fish muscle in its natural condition is an extremely complex material, both from chemical and physical standpoints. Any procedure such as disintegration of muscle fibers by blending is likely to have a marked effect on subsequent changes taking place in the muscle, including lipid oxidation. This is shown in the following experiment.

Similar cod fillets were divided into two groups. Homogenates were prepared from the first group in the usual manner, adding Cu^{++} after blending. With the second group, Cu^{++} was added directly to the water used for blending. In both cases the final concentration of Cu^{++} in the homogenates was 20 ppm. They were stored immediately at 0°C and examined after 24 hr with the following results:

Treatment	*TBA Value*	*Odour*
Cu^{++} added before blending	0.045	Very slightly seaweedy
Cu^{++} added after blending	0.61	Rancid

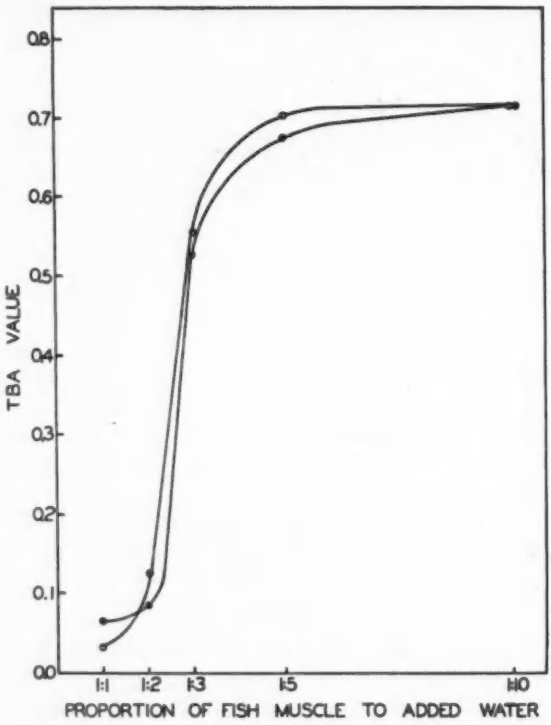


FIG. 6. Effect of fish:water ratio in the muscle homogenates on the development of rancidity during 24 hr storage at 0°C (duplicate samples having a final Cu++ concentration of 20 ppm).

Repetition of the experiment brought similar results. Addition of Cu^{++} to the homogenate after blending always resulted in very much higher TBA values than those that developed when the Cu^{++} was added directly to the blending water.

As blending is one of the factors in this procedure, it was considered desirable to determine the effect of varying the blending time, in addition to adding Cu^{++} before and after blending. Three homogenates were prepared with Cu^{++} added directly to the blending water with blending times of 15, 45, and 120 sec, respectively. Homogenates were also prepared and blended for the same times but the copper was added after blending. Figure 7 shows the TBA values after storage for 24 hr at 0°C. Increasing the blending time resulted in slightly increased rancidity when Cu^{++} was added subsequent to blending. On the other hand, increasing the blending time with the Cu^{++} in the blending water caused a very rapid decrease in the development of rancidity as indicated by both odour and TBA value.

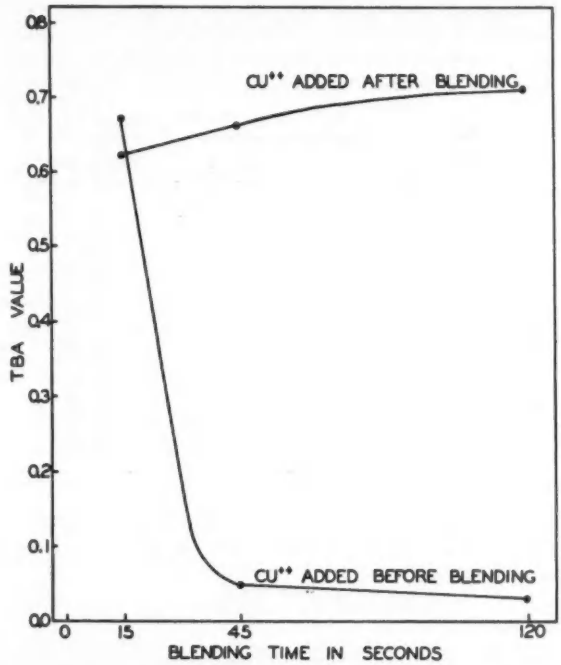


FIG. 7. The effect of blending time on the development of rancidity in two cod muscle homogenates; one had Cu++ added before blending and the other after blending.

Another variation in this experiment was carried out by using various concentrations of Cu^{++}, in addition to blending before and after adding Cu^{++}. The results showed that when the Cu^{++} concentration was increased above 25 ppm the phenomenon described above ceased to exist, and those blended before the addition of the Cu^{++} were equally or more rancid than those blended after.

EFFECT OF DIFFERENT COPPER SALTS

The results of experiments with different copper salts indicate that the catalytic action is dependent upon the amount of copper ions that are present. For example, Table II shows the TBA values at 24 hr for cod muscle homogenates to which eight different copper salts were added. The concentrations used were those that would give 20 and 30 ppm of Cu^{++} from the completely dissociated salts. The pH values for the homogenates were approximately 6.7 and they were not affected by these salts in the amounts added. It can be seen that the TBA values for the fish to which the dissociated salts were added are all the same. The copper oxide is extremely insoluble and the copper citrate undergoes very little dissociation. Therefore these two salts will have little or no copper ion present to act as a catalyst.

TABLE II. The effect of eight different copper salts on the development of rancidity, as indicated by TBA value, in cod muscle homogenates after 24 hr at 0°C.

	TBA value	
Salts	20 ppm Cu++	30 ppm Cu++
Copper nitrate	0.49	0.54
Copper carbonate	0.47	0.52
Copper sulphate	0.43	0.53
Copper chloride	0.40	0.52
Copper acetate	0.40	0.52
Copper hydroxide	0.27	0.51
Copper citrate	0.02	0.02
Copper oxide	0.02	0.02

These results suggest that as long as the copper salt is soluble and readily dissociated it may be used as the catalyst in this accelerated test for studying the development of rancidity.

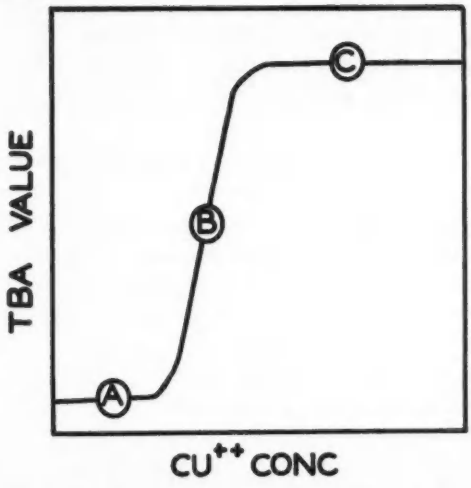
AVAILABLE OXYGEN

If the copper-treated homogenate is stored in an atmosphere of purified nitrogen gas, rancidity will not develop. On the other hand, if the homogenate is stored in a tall glass column with its surface exposed to the atmosphere, the rancidity develops faster at the top of the column. These and other similar observations indicate the importance of atmospheric oxygen to the reactions involved, and one might enquire whether the whole technique would not be improved by having better control over the amounts of oxygen entering into the reactions. So far this has not been found necessary if the following precautions are taken: duration of blending must be carefully timed for 45 sec; the flasks containing the homogenates must always be the same size so that the surface area and the depth of the homogenate are the same in every case; care must be taken to mix the homogenate thoroughly immediately before weighing out an aliquot for chemical testing; and last, a series of tests are never made by taking periodic samples from a single flask of homogenate. Each individual test is made from one flask which is discarded immediately after being sampled.

SUMMARY AND DISCUSSION

The results reported in this paper have shown that homogenates prepared from cod or haddock muscle to which trace amounts of copper ions have been added, followed by storage at 0°C, will develop a series of rancid odours and that the development of these odours is closely correlated with the TBA values of the stored fish.

When the TBA values are plotted against the concentration of added copper ions for a given storage period, or when they are plotted against different storage periods for a single concentration of copper ions, the result is a characteristic curve similar to that shown in Fig. 8. This curve has three distinct phases, shown as A, B, and C.



The first portion of the curve (A), in which there is little or no increase in TBA values, represents the induction period, followed by the second phase (B), during which the lipids undergo rapid oxidation. The factors that determine where this levels off at C have probably to do with the original amount of unsaturated lipids in the fish muscle. For the purpose of following the development of oxidative rancidity we are principally concerned with the A and B portions of the curve.

In order to get the position and slope of the line B, a series of points are required. These can be obtained by using either time in storage or copper ion concentration as the variables. The required minimum number and the distribution of these points will vary with the nature of the experiment. It has been found for most of our work that samples of homogenate containing 20 ppm Cu^{++} and stored for 0, 24, 48, and 72 hr gave sufficient data. An alternative, and frequently more convenient procedure, was to use 0, 5, 10, 20, and 30 ppm Cu^{++}, stored for a period of 24 hr.

It will be shown in subsequent papers in this series that the rate at which muscle lipids are oxidized varies slightly from one fish to another; it varies considerably from season to season; it is also affected by changes that occur in fish during frozen storage. It is not possible, therefore, to set up standard curves for fish muscle that will be consistent throughout the year. In each separate experiment dealing with factors affecting the development of rancidity, the standard for comparison is always the control that has been prepared from the same original homogenate.

Outline of Procedures

In brief, the procedures recommended on the basis of present experience are as follows:

1. Use fresh fish of good quality, carefully cutting out the fillets under aseptic conditions so as to have the lowest possible bacterial contamination.

2. Blend the whole fillets with twice their weight of distilled water. We use a gallon-size Waring blendor running at low speed for 45 sec.

3. Distribute this blended fish in 75-g amounts in clean 250-ml Erlenmeyer flasks.

4. Add 25 ml of copper solution so as to give the required concentration of Cu^{++} in parts per million in the homogenate.

5. Stir by vigorous rotary motion to thoroughly mix the copper solution and the fish muscle homogenate. (The efficiency of the mixing can be seen by examining the flask under ultraviolet light in a dark room; the copper combines with the proteins in the fish to give an orange-pink fluorescence. However, this should not be a regular procedure.)

6. It is usually necessary to scrape down any blended fish material adhering to the neck or sides of the flasks.

7. Place immediately in cold room at 0°C.

8. After the required holding period has elapsed, first examine for odours, then stir well and remove 40 g of the homogenate (which contains 10 g of fish muscle) for pH determination and the TBA determination.

9. The procedure used for the TBA determination is that given by Tarladgis *et al.* (1960), which is also described in the experimental procedures in this paper.

10. Each TBA determination is made on a separate flask of homogenate.

11. For most purposes several different Cu^{++} concentrations, ranging between 0 and 50 ppm are used (most commonly 0, 10, 20, and 30 ppm Cu^{++}); or one concentration, 20 ppm, is used for tests made on at least 3 successive days.

Limitations and Accuracy of the Technique

In order to give some idea of the accuracy and reproducibility of the values obtained by this technique for studying rancidity, the experiment summarized in Table III was carried out.

By inspection it can be seen that the reproducibility of the results is consistent over the range of TBA values that were encountered. Experience has shown that under certain conditions the degree of accuracy suggested above cannot be obtained without special care. This refers particularly to TBA values measured during the period of very rapid increase in rancidity following the induction period. Unless special care is taken in timing the tests, the results will show greater variations than similar tests made at an earlier period or after the curve has leveled off.

TABLE III. Experimental data showing accuracy and reproducibility of the procedure used for studying Cu++ catalyzed (20 ppm) rancidity in cod muscle[a].

Sample No.	TBA value			
	I	II	III	
1	0.520	0.44	0.73	0.73
2	0.525	0.50	0.70	0.69
3	0.535	0.58	0.65	0.65
4	0.525	0.40	0.61	0.62
5	0.535	0.38	0.58	0.57
6	0.490	0.48	0.49	0.50
7	0.540	0.52	0.25	0.18
8	0.545	0.55	0.06	0.12
9	0.540	0.45	0.10	0.08
10	0.550	0.49	0.03	0.03
Mean:	0.530	0.479		
Standard deviation	0.0162	0.060		

[a]Column I: TBA values from 10 replicate tests made from homogenate obtained from one fillet. Column II: TBA values of homogenates made from 10 similar fillets. Column III: TBA values from duplicate samples of 10 different fillets purposely prepared to give a wide range of values.

The chief purpose of this accelerated rancidity technique is for studying the effect of natural and artificially controlled factors on the development of rancidity in fish muscle. There is one phase that obviously cannot be studied without making some basic change in the technique. This includes the effect of the heme pigments and other pro-oxidants in the tissues. The addition of the active copper catalyst will mask the effect of variations in rancidity normally brought about by these less active pro-oxidants.

REFERENCES

CASTELL, C. H., JACQUELINE DALE AND N. DAMBERGS. 1962. Non bacterial spoilage in Atlantic ground fish: metal induced rancidity. *Canadian Fisherman*, **49**(9): 30–33.

DYER, W. J., AND DORIS I. FRASER. 1959. Proteins in fish muscle. 13. Lipid hydrolysis. *J. Fish. Res. Bd. Canada*, **16**(1): 43–52.

TARLADGIS, B. G., BETTY M. WATTS, MARGARET T. YOUNATHAN AND LEROY DUGAN, JR. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chemists' Soc.*, **37**: 44–48.

Rancidity in Lean Fish Muscle. II. Anatomical and Seasonal Variations[1]

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ABSTRACT

In a study of copper-catalyzed rancidity of cod fillets it has been found that: (1) muscle from the tail section goes rancid faster than muscle from the head or centre sections; (2) there is no apparent difference in the development of rancidity in the inner and outer halves of fillets that have been sliced lengthwise; and (3) sensitivity to rancidity changes with the season and is greater in the winter and early spring than in the summer and fall.

INTRODUCTION

It is known that muscle samples taken from different portions of the fillet are not chemically identical; it is also known that muscle taken from fish during various phases of the feeding and spawning cycles is subject to a considerable variation in the chemical composition. This applies particularly to the lipid content and to some of the other constituents of muscle that directly affect the lipids.

With this in mind, the accelerated copper-catalyzed test (MacLean and Castell, 1964) has been used to determine whether muscle taken from different areas of the fillet, and whether fillets taken from fish caught at different periods of the year, show any measurable difference in the rate at which they develop oxidative rancidity.

EXPERIMENTAL PROCEDURES

All tests were carried out on the muscle of cod (*Gadus morhua*). The procedures used for measuring the extent of rancidity in the muscle have been described in a previous paper (MacLean and Castell, 1964).

EXPERIMENTAL RESULTS

Rancidity in Muscle Taken from Different Areas of the Fillet

In preliminary tests it had been observed that if whole-gutted fish or whole fillets were immersed in weak solutions of copper salts and then stored at 0°C they became rancid, but the odours did not develop uniformly over the whole surface of the fish or fillet. They developed sooner and were stronger in the tail section of the fillet. Occasionally the thin belly flap also became rancid before the thicker muscle at the middle or head end of the fillet. It was possible to reduce the concentration of Cu^{++} in the solution to a level where rancid odours only developed in the tail section of the fish or fillet.

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Under ultraviolet light, cod muscle freshly treated with copper ions showed a rosy pink fluorescence. In isolated areas where rancidity had developed, the colours changed from pink to yellow, and at a more advanced stage, from yellow to blue. These observations of colour changes under ultraviolet light agreed well with development of rancid odours in isolated areas of the fillets.

Sections of cod fillet cut from the head, centre, and tail areas (Fig. 1A) were tested separately for their sensitivity to copper-catalyzed rancidity. It was found that the tail section was invariably the first to go rancid, followed by the head and then the centre (Fig. 2). Running lengthwise along the exterior side of the fillet there is a band of dark muscle. Along the centre line it dips deeply into the white muscle (Fig. 1B) but spreads out quite thinly over the remaining surface of the fillet. It forms a proportionally greater part of the fillet at the tail end than at the head or centre. Fraser *et al.* (1961) found that in haddock, similar to cod, the dark muscle makes up about 8% of the tail section but only 1% of the middle section.

Comparison was made of the sensitivity to copper-catalyzed rancidity of the white and dark muscle in cod fillets. These were cut from very large fish in order to get sufficient quantities of the dark muscle with which to work. The individual fillets weighed between 3.2 and 3.6 kg. The dark muscle was approximately 3% of the whole fillet. The total lipid in the white muscle was 0.60–0.74% and in the dark muscle was 1.88–2.06%. In each of the specimens examined, the dark muscle had a higher free fatty acid content than the white

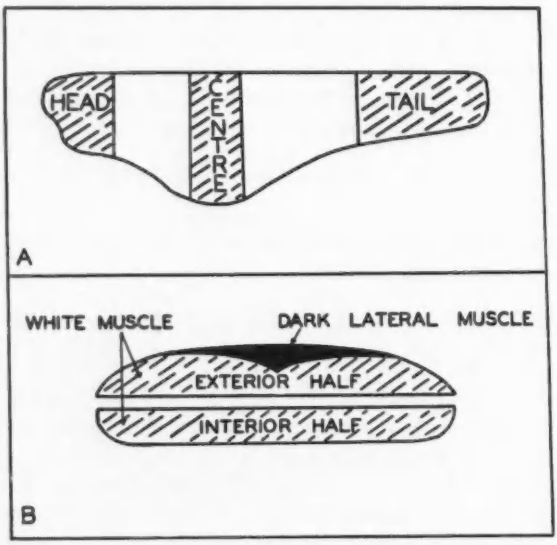

FIG. 1. Areas of cod fillet used in tests. A. The head, centre, and tail sections cut from the whole fillet. B. Cross-section of the fillet that has been split lengthwise. The dark lateral muscle is located on the exterior or skin side of the fillet.

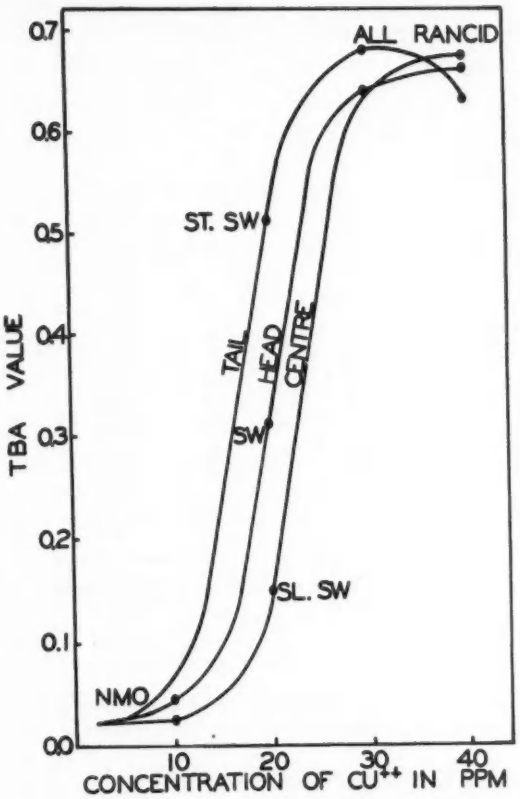

FIG. 2. TBA values of homogenates made from head, centre, and tail sections of cod fillets to which 10, 20, 30, and 40 ppm Cu++ had been added and stored for 24 hr at 0°C. (NMO — not much odour; SL.SW — slightly seaweedy; ST.SW — strongly seaweedy.)

muscle. Experimental results, shown in Fig. 3, demonstrate that the dark muscle not only went rancid faster but also produced higher maximum thiobarbituric acid (TBA) values.

Tests were also made, using the white muscle only, from the head, centre, and tail sections of the fillet. Again it was found that the tail end was the first to go rancid. This would indicate that the greater sensitivity of the tail portion of the whole fillet is not only the result of its greater proportion of dark muscle.

A number of fillets were cut lengthwise, dividing them into exterior and interior portions (Fig. 1B). In spite of the fact the exterior half contained almost all of the dark muscle, there was no significant difference in the rate of the development of rancidity in the homogenates prepared from the inner and outer portions. Although the dark muscle contains about three times as much

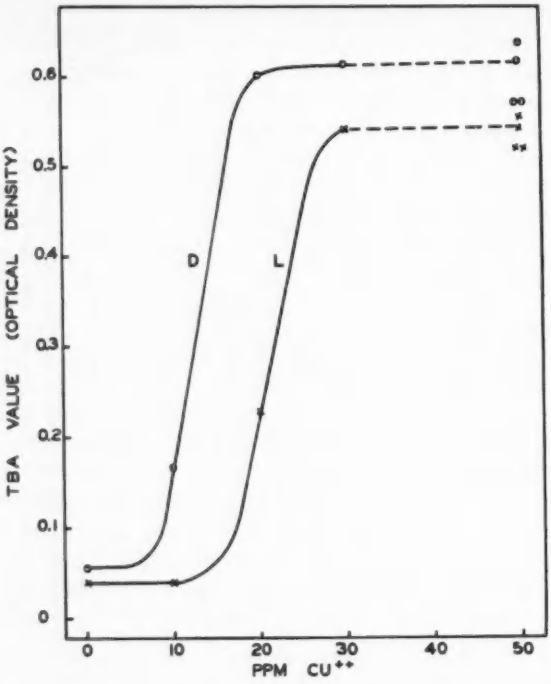

FIG. 3. Development of copper-induced rancidity in light (L) and dark (D) muscle of cod fillets stored 24 hr at 0°C.

lipid as the light muscle, the total amount of dark muscle is only a small fraction of the whole fillet.

EFFECT OF SEASON

During 1963 and January of 1964, 184 tests were made on homogenates prepared from individual fillets, to which 20 ppm Cu^{++} had been added and stored for 24 hr at 0°C. The resulting TBA values that occurred throughout the year are shown in Fig. 4. During January, February, and March, when the fish were in prime condition, the lipids were quite sensitive to oxidation. There was a very definite decrease in this sensitivity for many of the fish tested during June and July. During late summer and fall, the values remain quite scattered with many showing a continued resistance to rancidity. In January the values increase again to complete the cycle.

As pointed out previously (MacLean and Castell, 1964), data based on single points of the TBA storage time curves do not give a very complete picture. The lower summertime values, for instance, do not show whether the oxidation has been prevented or merely retarded for a short period. A better comparison of fish from different seasons is given in Fig. 5. Here the TBA values for homogenates from 10 spring and 10 summer fillets are plotted against hours at

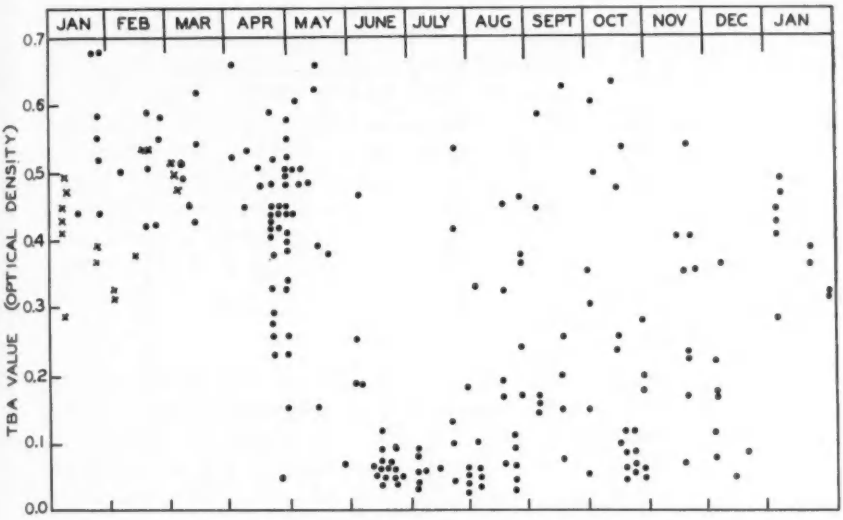

FIG. 4. Seasonal variation in the susceptibility of cod muscle to copper-catalyzed rancidity. These fish were caught off the east coast of Nova Scotia and on the Grand Banks during January 1963 to February 1964. The Xs at the left show results obtained during 1964.

0°C. The concentration of added Cu^{++} was 20 ppm. It can be seen that the chief difference is the short induction period for the spring-caught fish. This induction period has been delayed approximately 24 hr for the summer-caught fish. Once the fats begin to oxidize rapidly the rates are approximately the same. The range within which the TBA values level off (i.e. approximately 0.65) is the same for both spring- and summer-caught fish. This suggests the presence of additional amounts of antioxidants in the summer fish and could well be associated with the voracious feeding that follows the periods of spawning and fasting that occurs chiefly during the spring in this area of the Atlantic.

FISH FROM MORE NORTHERN WATERS

Up to this point all tests recorded in these investigations have been carried out with fillets cut from fish caught on the "Western" and "Grand Banks" of the Atlantic. During the summer months fish from other areas become available. In June and July some of the trawlers fished in the more northern Strait of Belle Isle area, between Newfoundland and Labrador. This area has a different fish population that spawns much later than the fish in the more southern areas. It seemed of interest, therefore, to run tests on fish from both areas and determine whether similar seasonal changes occurred in fish from the different populations. During the first week in July 1963, tests were made at the same time on fillets

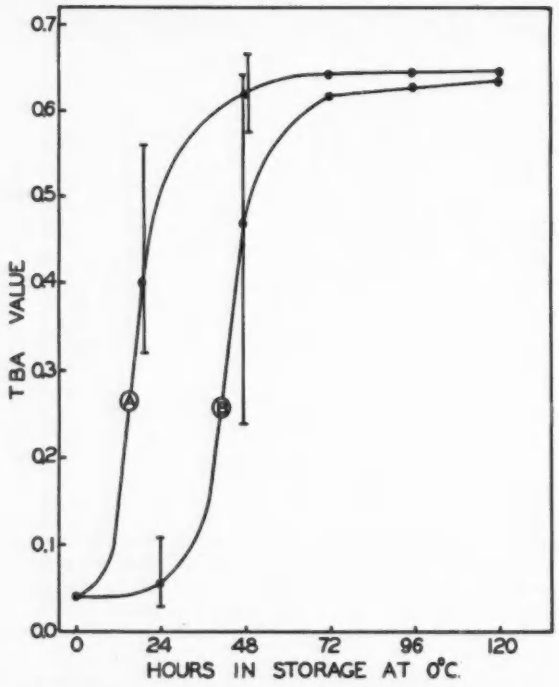

FIG. 5. Mean TBA values for copper-treated homogenates prepared from cod caught about the middle of April (A), and the middle of June (B). The perpendicular lines show the range of TBA values for 10 different samples.

cut from Grand Bank fish and fish of the same size and freshness from the Strait of Belle Isle. At 24 hr, with 20 ppm Cu^{++} added, the results were as follows:

	Grand Banks	*Strait of Belle Isle*
Number of fish tested	22	8
Mean TBA value	0.06	0.54
Range of TBA values	0.03–0.11	0.24–0.63
Odours	None to very slight seaweedy	Seaweedy to rancid

This experiment was repeated with similar small numbers of fish from both areas, and approximately the same odours and TBA values were obtained.

If these are truly representative samples, it would indicate that the fish from the more northern waters are similar in June and July to the fish from the Grand Banks in April or early May. Of course, what this similarity is, and what causes it, remains to be determined.

Size of Fillets

During the month of April 1963, 54 cod fillets ranging in weight from 137 to 802 g were made into homogenates and tested in the usual manner, adding 20 ppm Cu^{++} and storing for 24 hr at 0°C. Although there were considerable variations in the TBA values, they do not show any trend which would suggest a correlation between fillet size and susceptibility to the development of rancidity. Whether this is true for fish during other periods of the year has not yet been ascertained. It was unfortunate that these tests to determine the effect of size were inadvertently carried out in the period during which the fish were also undergoing marked changes in sensitivity to the development of rancidity from other, unknown causes.

SUMMARY AND DISCUSSION

1. The results of these experiments with cod show that all portions of the fillet are not equally susceptible to copper-catalyzed rancidity. Under similar conditions the rancidity always developed earliest in the muscle from the tail section of the fillet (i.e. posterior to the gut cavity).

The chemical composition of fish muscle varies in different regions of the fillet. This applies particularly to the lipid content and to some of the other constituents of the muscle that directly affect the lipids. Dambergs (1963) has shown that the tail sections of cod fillets have about 25% more lipid than other portions of the fillet. Fraser *et al.* (1961) found for cod and haddock that the dark lateral band of muscle contains 2% lipid compared to 0.7–0.8% for the lighter muscle. They have also shown that in cod and haddock this darker muscle makes up 8% of the flesh in the tail section and only 1% of the middle section of the fillet. Fraser *et al.* (1961) have also pointed out that Braekkan (1955) had previously described some of the differences in the composition of the red and white muscle taken from fish, suggesting that the dark muscle may act as a store house for fat, glycogen, and other metabolites in close proximity to the ordinary white muscle. Bligh (unpublished data), on the other hand, found only quantitative differences in the lipids in the light and dark muscle of these fish, and the various lipid fractions in approximately the same proportions were common to both. Namiki (1934), and many other workers since then, have shown that the dark muscle of fish contains more organically bound iron than the ordinary white muscle. These hematin compounds catalyze the oxidation of unsaturated fats, predisposing the dark muscle to more rapid development of rancidity.

There is little doubt that with untreated cod fillets, held under conditions permitting oxidation of the lipids, rancidity will develop first in the dark lateral muscle. It has more fat and is richer in hematin compounds that catalyze lipid oxidation. By adding an excess of the more active copper-catalyst it has been shown that apart from differences in the hematin, the fat in the dark muscle is more subject to oxidation than that of the white muscle.

It has also been shown that the white muscle in the tail section of the fillet is more sensitive to copper-catalyzed rancidity than the white muscle from the

centre or head sections. These observations would suggest that some interesting difference might be found in the function or composition of the lipids in the tail section compared to the other portions of the fillet. In this connection it is interesting to note the recent observations of Dambergs (1964), that whereas the lipid content of the head and centre portions of cod fillets undergoes marked changes with the spawning and feeding cycles, that of the tail section remains relatively constant. It could well be that the lipid in tail portions is more directly associated with the constant motion of the fish, acting as a ready source of energy. While at the opposite end it has the additional function of energy storage for the whole organism.

2. It has also been shown that fillets cut from fish caught at different periods of the year are not equally affected by the addition of trace amounts of Cu^{++}. Rancidity develops faster in the winter-caught fish than in those taken in the summer. The transition period is in late April and in May. During fall the values for individuals tend to scatter with the majority remaining more resistant to rancidity. Once winter (January) has set in, the cycle begins again.

From the few results that are available it is of interest to note that fish taken in July from the northern waters of the Strait of Belle Isle and off Labrador did not show the retarded rancidity characteristic of the fish taken from the banks off the east coast of Nova Scotia at this time. These fish are from different populations and those from the more northerly areas are known to spawn many weeks later. It would be of interest to follow fish from both areas throughout a whole season, and see whether the fish off Labrador produced a similar picture with changes occurring at a later period than those from the western banks.

The TBA–Cu^{++} concentration curves suggest that the seasonal differences are primarily concerned with the induction period in the development of rancidity. This could be the result of differences in the natural antioxidants in the muscle, particularly tocopherol, which are known to fluctuate with the feeding cycle of the fish. It is also of interest to note that DeWitt (1963), working with cod livers, found that the iodine value varied with the spawning and feeding cycles. For northern European waters, maximum values were found to occur in the winter, followed by an abrupt fall in March when the cod spawns. The unsaturation slowly increased during the summer period of intensive feeding.

3. The initial purpose of these particular experiments was to determine whether normal variations in fresh cod muscle would be sufficient to interfere with the tests for rancidity using measured amounts of copper in homogenized muscle held for 24 hr at 0°C. The results have indicated the need for certain precautions. It is better to blend whole fillets in preparing the homogenates, even though smaller portions of the fillet might provide enough homogenate. In this way one can eliminate variations in susceptibility that occur in different portions of the fillet.

It is also obvious that results from experiments performed at one period of the year cannot always be duplicated at other periods. In June or July, for example, it is necessary to use a greater concentration of Cu^{++}, or to store the

homogenates for longer periods to get values that are comparable to those found in January or February. It is for this reason that standard curves for rancidity as measured by TBA values cannot be prepared. In each individual experiment dealing with factors that affect the development of rancidity, the standard for comparison must always be the controls that have been prepared at the time the experiment is being carried out.

REFERENCES

BRAEKKAN, O. R. 1958. Fiskeridirektoratets Skrifter, Serie Teknologiske Undersøkelser, Pt. 3, **3**:(6): 1–32.

DAMBERGS, N. 1963. Extractives in fish muscle. 3. Amounts, sectional distribution and variations of fat, water-solubles, protein and moisture in cod (*Gadus morhua* L.) fillets. *J. Fish. Res. Bd. Canada*, **20**: 909–918.

1964. Extractives in fish muscle. 4. Seasonal variations in fat, water solubles, protein and water in cod (*Gadus morhua* L.) fillets. *J. Fish. Res. Bd. Canada*, **21**: 703–709.

DEWITT, K. W. 1963. Seasonal variations in cod liver oil. *J. Sci. Food Agric.*, **14**: 92–98.

FRASER, D. I., A. MANNAN AND W. J. DYER. 1961. Proximate composition of Canadian Atlantic fish. III. Sectional differences in the flesh of a species of Chrondrostei, one of Chimaerae and of some miscellaneous teleosts. *J. Fish. Res. Bd. Canada*, **18**(6): 893–905.

MACLEAN, JILL, AND C. H. CASTELL. 1964. Rancidity in lean fish muscle. I. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. *J. Fish. Res. Bd. Canada*, **21**: 1345–1359.

NAMIKI, S. 1934. Über den Kreatin-Kreatinin-Eisen-und-Fettgehalt der Muskulatur von Fischen. *Nagoya J. Med. Sci.*, **7**(3): 206–207.

Rancidity in Lean Fish Muscle. III. The Inhibiting Effect of Bacterial Activity[1]

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ABSTRACT

Actively growing bacteria suppress the development of copper-catalyzed rancidity in lean fish muscle. Apart from the antioxidant effect of the bacteria themselves, it could not be shown that fish muscle undergoing microbial deterioration became any less susceptible to oxidative rancidity as spoilage progressed.

INTRODUCTION

It has been the general experience in work dealing with fatty foods that actively growing bacteria tend to inhibit the development of oxidative rancidity. Fish are no exception. Lerke (1961), working with rockfish and salmon, found that when microbial spoilage was inhibited by radiation or antibiotics, oxidative rancidity developed. Stansby (1962) stated that it was his own experience, as well as the experience of investigators at the Torry Research Station in Scotland and the Low Temperature Research Station at Cambridge, that as long as bacterial spoilage was taking place, oxidative rancidity did not develop.

This paper is concerned with two aspects of the relationship between bacteria and the development of copper-induced rancidity in cod muscle (*Gadus morhua*). First, the extent to which bacteria will retard rancidity; second, to determine whether there is any change in the susceptibility of cod muscle to copper-induced rancidity as it undergoes the earlier stages of microbial spoilage.

EXPERIMENTAL PROCEDURES

The methods used for preparing the cod muscle homogenates, adding the copper catalyst, storing the samples and measuring the thiobarbituric acid (TBA) values were the same as those described in the first paper in this series (MacLean and Castell, 1964).

The effect of bacteria on the development of rancidity was observed under two different conditions: (1) where heavy suspensions of pure cultures of bacteria were added directly to the cod muscle homogenates and then stored at 0°C; (2) where the growth of the normal bacterial contaminants from the fillets was accelerated by storing the homogenates at 25, 37, and 56°C instead of the usual 0°C. In addition, the effect of bacterial cells killed by heating to 100°C for 15 min was also examined.

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In the second portion of the work, tests were made to determine whether the extent of bacterial deterioration that had already taken place in the muscle would affect the subsequent development of copper-induced rancidity. To do this, advantage was taken of the fact that normally the flesh of freshly killed fish is free from bacteria and that during the earlier stages of spoilage most of the bacterial activity is confined to the surfaces of the fish or in the gut cavity. It is possible, therefore, to cut flesh from the interior of spoiling fish muscle that is almost completely free from bacteria. In these experiments fish were allowed to deteriorate as whole fish and also as excised fillets. Periodically, fillets were cut from the deteriorating whole fish under conditions that kept bacterial contamination at a minimum. For comparison, the fish that spoiled as fillets were blended without any attempt to remove the very large numbers of bacteria growing on their surface.

The bacterial plate counts and the methylene blue reduction test were carried out according to standard methods and the trimethylamine values were obtained by the method of Dyer (1945, 1950).

EXPERIMENTAL RESULTS

Effect of Bacteria on the Development of Rancidity

In the first test heavy suspensions of a psychrophilic *Achromobacter*, isolated from cod fillets, were added to cod muscle homogenates containing 0, 10, 20, 30, and 40 ppm Cu^{++} and then stored at 0°C. After 24 and 96 hr the odours were recorded and the TBA values determined. At the same time a similar series of tests was carried out using heat-killed cells. Figure 1 shows the TBA values and odours that developed in the samples containing 20 ppm Cu^{++} and which are typical of the others in the series. It can be seen that the live cells suppressed the development of rancidity. The homogenates containing the live cells did, however, develop strong off-odours and subsequently became putrid through bacterial action. The addition of heat-killed cells did not suppress the development of rancidity.

Similar experiments were carried out with other species of bacteria. The psychrophilic cultures had a much greater inhibiting action than those organisms that normally grow at higher temperatures. Thus, *Escherichia coli* and other "blood heat" organisms had little or no effect at 0°C.

In the second set of experiments the normal microflora from the fillets was allowed to increase by raising the storage temperatures. For example, homogenates to which 20 ppm Cu^{++} was added were stored at 0, 10, 25, 37, and 56°C. After 24 and 48 hr their TBA values and bacterial counts were determined (Fig. 2A and 2B). At 25 and 37°C, where the bacteria in the muscle grew very rapidly, the TBA values were depressed. At 0 and 10°C, where bacterial growth was very slow, the TBA values increased rapidly. At 56°C the bacteria were killed and there was a very rapid increase in TBA values. It is interesting to note that at 56°C, after the maximum TBA values had been reached, instead of leveling off, the values went down, suggesting that the compounds responsible for the colour reaction are decomposed at this temperature.

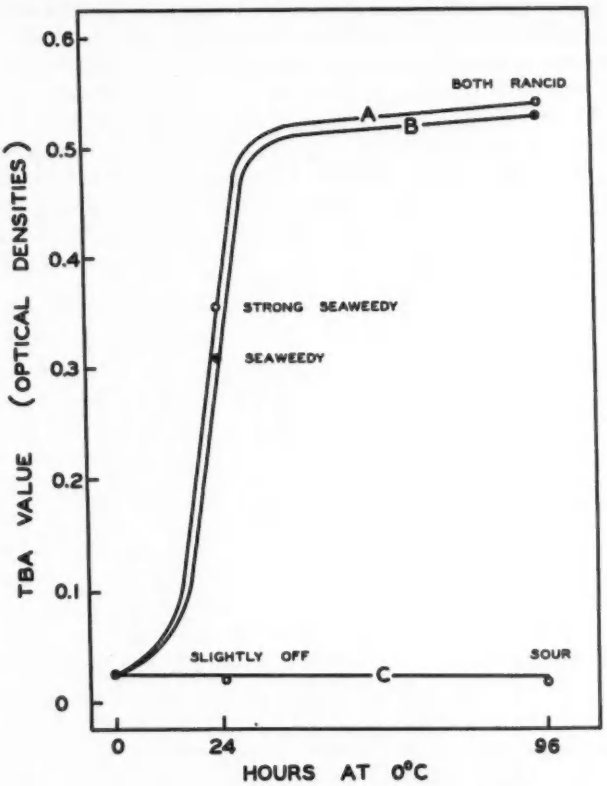

FIG. 1. Effect of adding heavy suspensions of (A) heat-killed and (C) actively growing cells of Achromobacter No. 176 to fish muscle homogenate stored at 0°C and containing 20 ppm Cu^{++}. Line (B) is the control to which no bacteria were added. The figures show the TBA values and odours at 24 and 96 hr storage time.

In a somewhat similar experiment, homogenates containing 20 ppm Cu^{++} were stored at 0, 10, 25, 37, and 56°C. At 24 hr the odours and bacterial counts were recorded and the reduction times for added methylene blue (similar to the standard test used in dairy bacteriology) were measured (Table I). The development of the strong seaweedy odours coincided with low bacterial counts and very long methylene blue reduction times.

These and other experiments all indicate that where bacteria are *growing rapidly* in the fish muscle, high TBA values and rancid odours do not develop, even in the presence of Cu^{++}. The mere presence of bacterial cells, even in very large numbers, under conditions where growth is stopped is not sufficient to inhibit the rancidity.

It was also observed that once the fish muscle has become strongly rancid the growth of many species of bacteria is stopped or retarded. This bacteriostatic action is beyond that which can be accounted for from the metal ions themselves.

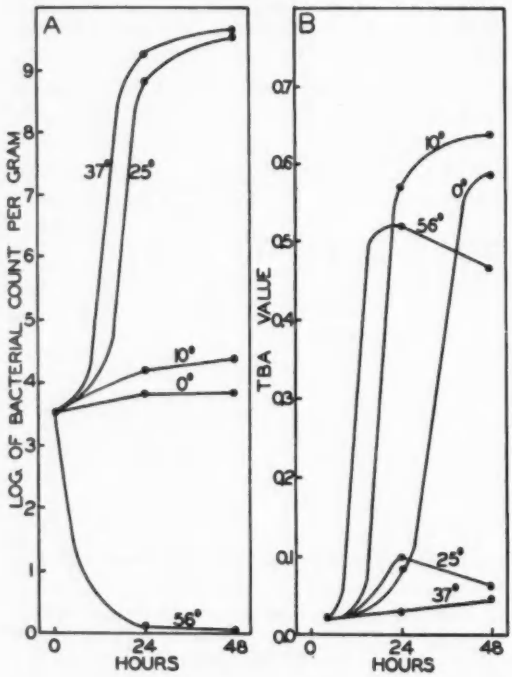

FIG. 2. Bacterial counts (A) and TBA values (B) for blended cod muscle to which 20 ppm Cu++ had been added and then stored at 0, 10, 25, 37, and 56°C.

TABLE I. Bacterial plate counts, odours, and methylene blue reduction time of cod muscle homogenates to which 20 ppm Cu++ had been added and then held at five different temperatures for 24 hr.

Holding temperature (°C)	Bacterial plate counts per gram at 25°C	Odour of fish homogenate	Methylene blue reduction time at 25°C
0	22,000	St. seaweedy	More than 5 hr
10	40,000	St. seaweedy	More than 5 hr
25	120,000,000	Sour fruity	Less than 1 min
37	450,000,000	Putrid-fecal	Less than 1 min
56	less than 1,000	Fish chowder and seaweedy rancid	More than 5 hr

It was also interesting to note that in those instances where vigorous bacterial growth followed the development of this copper-induced rancidity, the compounds responsible for the TBA reaction were apparently unaffected. An example of this was shown in fig. 5 of the first paper in this series (MacLean and Castell, 1964). Twenty and 50 ppm Cu^{++} were added to cod muscle homogenates stored at 0°C. They quickly became strongly rancid and had high TBA values. Between 10 and 11 days bacterial odours also began to develop and soon became so strong that they masked the rancidity. By 30 days the fish was badly decomposed and smelled "putrid", but there was little or no reduction in the TBA values.

Effect of Bacterial Deterioration on TBA Values and the Subsequent Development of Rancidity

During the past 3 years TBA determinations have been made on a great many samples of cod and haddock muscle undergoing various stages of bacterial decomposition. So far there is nothing in our results to indicate that bacterial disintegration of the fish muscle is accompanied by increases in the TBA value.

Fresh fillets were stored at 0°C and tested periodically for indications of deterioration. Between the sixth and eighth day the trimethylamine (TMA) values began to rise sharply and this was accompanied by the first off-odours resulting from bacterial activity. By 13 days the TMA values had increased to somewhere between 16 and 20, and the fish had become offensive with strong fruity-sour odours. There was no increase in the TBA values as the fish underwent these earlier stages of spoilage. After 0, 6, 9, and 13 days some of these fillets were removed from storage and tested for their susceptibility to oxidative rancidity by homogenizing and adding copper and storing at 0°C in the usual manner. Figure 3 shows the series of TBA values and odours 24 hr after adding the Cu^{++}. For the first six days there is no apparent change in the sensitivity of the fillets to oxidation. Following this, however, there was a pronounced decrease both in the rancid odours and TBA values. This decrease in the susceptibility of the stored fillets to rancidity coincided with increased bacterial activity as shown by bacterial counts, TMA values, and the development of off-odours.

In order to avoid this antioxidant action of the spoilage bacteria themselves, further tests were carried out with deteriorating whole round and with gutted fish stored in ice. Special care was taken to prevent the transfer of bacteria from the surface of the fish to the freshly excised inner portion of the muscle. In this way it was possible to get samples of spoiled muscle containing relatively few bacteria. These were then tested for susceptibility to rancidity by adding Cu^{++}. The results with both round and gutted fish showed that as deterioration increased there was no change in its susceptibility to the copper-induced rancidity.

The so-called "bilgy" fish is another very offensive type of spoilage that occurs when fish are jammed firmly against wooden pen boards in vessels or against the sides or bottoms of wooden boxes in plants. Among other things,

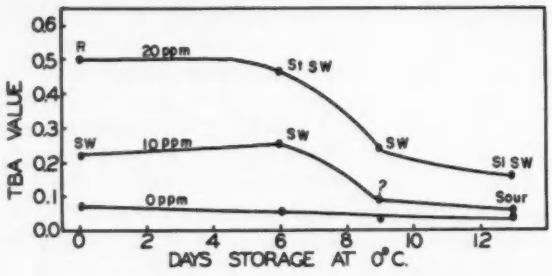

FIG. 3. Changes in the sensitivity to copper-catalyzed rancidity of cod fillets during 13 days storage. TBA values and odours are shown for homogenates containing 0, 10, and 20 ppm Cu^{++} after another 24 hr at 0°C. (SW—seaweedy; Sl SW—slightly seaweedy; St SW—strongly seaweedy).

this type of spoilage is accompanied by a relatively high hydrogen sulphide content in the muscle (MacLean and Castell, 1956).

Typical bilgy fish were obtained from a local trawler at the time of being discharged from the pens. At the same time, well-iced, non-bilgy fish were taken from the centre of the pens to serve as controls. These fish received no additional storage and were filleted on arrival at the laboratory. Immediately after filleting the following values were obtained as an indication of the quality of the fillets.

Spoilage indices	*Controls*	*Bilgy fish*
Odour	Fresh	Vile-bilgy
TMA (Mg TMA-N/100 g)	0.4	2.9
TBA (optical density)	0.07	0.03
H_2S content (mg/100 g)	21.0	118.0

Twenty ppm Cu^{++} was added to homogenates prepared from both lots of fillets and after 24 hr at 0°C the following observations were made:

	Control	*Bilgy fish*
Odour	Strong seaweedy	Rancid fish oil
TBA	0.42	0.58

During the period of storage with the added copper present in the muscle the bilgy odours completely disappeared. In addition to the tests using 20 ppm of added Cu^{++}, other tests were also made using 2.5 and 10 ppm Cu^{++}. In each instance where seaweedy or rancid odours developed, they were stronger in the samples prepared from the bilgy fish. Whether this is a constant characteristic of bilgy fish has not yet been determined; but from the results obtained, there is nothing to indicate that bilgy fish is any less sensitive to copper-induced rancidity than the unspoiled muscle from the control samples.

SUMMARY AND DISCUSSION

Bacterial deterioration of cod muscle at 0°C was not accompanied by increased TBA values nor the development of odours suggesting oxidative rancidity. This included spoilage of fillets stored at 0°C, spoilage of gutted and ungutted fish held in ice, and the vile, anaerobic type of spoilage that occurs when fish are jammed tightly against slime-soaked pen boards.

Rancidity in fish muscle to which copper had been added was stopped or strongly retarded by the addition of actively growing bacteria or by making conditions suitable for the rapid growth of the normal contaminants. Heat-killed cells did not possess this ability to suppress the development of rancidity.

Tests with pure cultures of bacteria have shown that fish muscle that has undergone copper-induced rancidity is a poorer substrate for bacterial growth than non-rancid muscle. This bacteriostatic action of the rancid fish is beyond what could be accounted for by the oligodynamic activity of the copper itself, and is not the same for all species that were examined.

Tests were also carried out to determine whether fish muscle, spoiling through bacterial action, became any less susceptible to copper-induced rancidity as the deterioration progressed. Where the muscle was cut aseptically from deteriorating whole fish or gutted fish there was no indication of decreased sensitivity. But fish, stored as fillets, apparently became less sensitive to oxidative rancidity as bacterial deterioration increased. It seems very probable, however, that this difference in susceptibility to rancidity, has to do with the large numbers of bacteria on the surface of the spoiling fillets rather than any chemical changes that might be involved in the muscle. In working with stored fillets, the bacteria were automatically incorporated into the blended homogenates where they were prepared. In the case of the whole or gutted fish, the spoilage organisms were mainly on the skin surface or in the gut cavity and were eliminated during the filleting of the fish.

REFERENCES

DYER, W. J. 1945. Amines in fish muscle. I. Colorimetric determination of trimethylamine as the picrate salt. *J. Fish. Res. Bd. Canada*, **6**(5): 351–358.

1950. Note on the colorimetric estimate of trimethylamine. *J. Fish. Res. Bd. Canada*, **7**(10): 576–579.

LERKE, P. A., L. FARBER AND W. HUBER. 1961. Preservation of fish and shellfish by relatively low doses of beta radiation and antibiotics. *Food Technol.*, **15**: 145–151.

MacLEAN, JILL, AND C. H. CASTELL. 1964. Rancidity in lean fish muscle. I. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. *J. Fish. Res. Bd. Canada*, **21**(6): 1345–1359.

McLEAN, NORMA L., AND C. H. CASTELL. 1956. Spoilage of fish in the vessels at sea: 5. Bilgy fish. *J. Fish. Res. Bd. Canada*, **13**(6): 861–868.

STANSBY, M. E. 1962. In the printed discussion following Chapter 9 (Marine Products) *in* Lipids and Their Oxidation, edited by H. W. Schultz, E. A. Day and R. O. Sinnhuber. The Avi Publishing Co., Westport, Connecticut.

Fatty Acid Distribution in Lipids of Marine Plankton[1]

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ABSTRACT

In the diatom, *Skeletonema costatum*, as well as in a zooplankton sample, the polyunsaturated fatty acids of the triglycerides were found accumulated in the β-position of the glycerol. This fatty acid distribution pattern is typical for animal fats, in particular for fish oils. Together with the recent demonstration that fish and invertebrates retain in part the structure of ingested triglycerides, the findings of the present study show that the typical structure of marine triglycerides originates in phytoplankton and is to a large degree retained through the marine food chains.

The fatty acid composition of several other lipid fractions of the plankton samples was also determined.

INTRODUCTION

Studies on marine fish (Brockerhoff and Hoyle, 1963) and invertebrates (Brockerhoff *et al.*, 1963) at this laboratory have shown that the triglycerides of these animals follow the structural pattern typical for most animal fats in that the polyunsaturated fatty acids are preferentially located in the β-position of glycerol. As a partial explanation of this phenomenon of specific fatty acid distribution, it was suggested that the typical triglyceride structure may originate in plankton and then be retained through the marine food chains. Explicitly, the hypothesis states that in the triglycerides of marine plankton the polyunsaturated fatty acids (mainly eicosapentaenoic, 20:5, and docosahexaenoic, 22:6, acid) are already predominantly bound to the β-hydroxyl group of glycerol, and that in the following stages of the marine food chains the triglycerides are not completely hydrolysed. Instead, they retain their β-monoglyceride structure, and the typical fatty acid distribution pattern in fish oils is therefore the continuation of a similar pattern in plankton, due to the fact that the β-monoglyceride linkages were never broken.

The hypothesis implies that those lipolytic enzymes of marine animals that assist in the absorption of fats should specifically split the α-bound, as against the β-bound, fatty acid esters of triglycerides. This was actually observed for the pancreatic lipase of a skate (Brockerhoff and Hoyle, unpublished data). Furthermore, direct evidence was obtained for a "retention of the β-monoglyceride structure". A triglyceride containing a labelled fatty acid in β-position was fed to lobster, cod, and trout, and the distribution of the label in the triglyceride of these animals was then determined. It was found that the invertebrate and

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the fish had retained 50–80% of the β-monoglyceride structure of the dietary triglyceride. These findings demonstrate the possibility of a retention of the β-monoglyceride structure through the marine food chains.

The present study is concerned with the lipids of marine plankton, specifically, with the question of whether plankton triglycerides do indeed possess the typical fatty acid distribution pattern as required by the hypothesis. A sample of mixed zooplankton and a culture of one of the most common diatoms, *Skeletonema costatum*, were investigated. The fatty acid compositions of some lipids other than triglycerides were also determined.

MATERIALS AND METHODS

Zooplankton was collected off Nova Scotia in late March 1963. As estimated by microscopic examination, one third of the sample consisted of fish eggs, one third of amphipods and the rest of copepods, isopods and annelids. The sample was dried by repeated evaporations with acetone and then extracted with chloroform–methanol–water (Bligh and Dyer, 1959), yielding 0.59 g lipid from 21.2 g dry material, or 2.8%.

As a representative of marine phytoplankton, a common diatom, *Skeletonema costatum*, was cultured as described elsewhere (Ackman *et al.*, 1964) and harvested by centrifugation; 43 g (wet) yielded 1.16 g or 2.7% lipid.

The lipids were separated on columns of silicic acid (Barron and Hanahan, 1958; Hanahan *et al.*, 1957), and the fractionation was followed by thin-layer chromatography (Vogel *et al.*, 1962). The positional distribution of fatty acids in triglycerides was determined by treatment with pancreatic lipase (Mattson and Volpenhein, 1962) followed by separation of the products of lipolysis on thin-layer silicic acid plates (Brockerhoff *et al.*, 1963). The fatty acid distribution in the lecithin from zooplankton was determined by hydrolysis with phospholipase-A from snake venom (*Crotalus atrox*) (Hanahan *et al.*, 1960).

The fatty acid composition of different lipid fractions was determined by gas chromatography of the methyl esters (Metcalfe and Schmitz, 1961) with an Aerograph Hy-FI Gas Chromatograph[2], equipped with a 12 × $\frac{3}{8}$-inch aluminum column with EGSS-X (ethylene glycol succinate–silicone polymer), 10% on 100–200 mesh Gas-Chrom P[3], as stationary phase. The carrier gas was nitrogen at 40 psi input pressure. Peak identification was based on log plot and separation factor procedures (Ackman and Burgher, 1963) and quantification on the assumption that the response of the hydrogen flame detector was proportional to the fatty acid chain length.

The fatty acid concentration in some lipid fractions was determined by titration after saponification. In certain fractions the identity and concentration of galactose was determined. A sample was hydrolysed with 5% aqueous HCl, and the products were separated by ascending paper chromatography with ethyl acetate–pyridine–water (Smith, 1960). The sugar was detected with alkaline $AgNO_3$ (Trevalyan *et al.*, 1950). The mobility, size, and density (visual)

[2]Wilkins Instrument & Research, Inc., Walnut Creek, California.
[3]Applied Science Laboratories, Inc., State College, Pennsylvania.

of unknown spots were compared with galactose, mannose, glycose, and fructose standards.

RESULTS

ZOOPLANKTON

The lipids of the zooplankton sample were fractionated on silicic acid with the following solvents:

Benzene–hexane (15:85, v/v) eluted hydrocarbons and sterolesters (Barron and Hanahan, 1958). Identification of this material (20% of the total lipid) was not attempted.

Hexane–ether (93:7, v/v) eluted triglycerides and free fatty acids. The fatty acids, 5% of the total lipids (perhaps artifacts), were removed by absorption on aluminum oxide and the triglycerides, 8% of the total lipids, were treated with pancreatic lipase. The fatty composition in α- and β-positions are given in Table I.

Chloroform presumably eluted sterols and partial glycerides, besides pigments, 12% of the total lipids.

Chloroform–methanol (80:20, v/v) eluted phosphatidyl ethanolamine, which was identified by comparison with egg yolk phosphatidyl ethanolamine on thin-layer chromatography (Vogel *et al.*, 1962) and the positive ninhydrine reaction. This fraction, 22% of the total lipids, may have also contained some phosphatidyl serine. The fatty acid composition is given in Table I.

TABLE I. Fatty acid composition of lipids from zooplankton in mole per cent.[a]

		Fatty acid										
	Position	14:0	16:0	16:1	18:0[b]	18:1	18:2	18:4	20:1	22:1	20:5	22:6
Triglyceride	α	9	21	14	4	23	4	2	6	3	2	1
	β	4	15	14	1	12	4	9	1	<1	15	11
Lecithin	α	5	45	6	3	14	1	4	2	1	11	4
	β	1	1	<1	<1	5	2	3	1	<1	36	41
Phosphatidyl-ethanolamine		2	15	<1	3	7	2	4	1	1	26	30

[a] Fatty acids present in less than 2% are omitted.
[b] Together with 16:4.

Chloroform–methanol (40:60, v/v) eluted lecithin, 32% of the total lipids, which was subjected to phospholipase-A hydrolysis. The fatty acid composition in the α- and β-positions of lecithin is given in Table I.

Methanol, the last solvent, eluted only 0.3% of the starting material.

The major phospholipids of this plankton sample were lecithin and phosphatidyl ethanolamine. Some sphingomyelin, identified by thin-layer chromatography, accompanied the lecithin. Whether plasmalogens were present was not determined. However, in the transesterification method used for the preparation of methyl esters (Metcalfe and Schmitz, 1961), plasmalogens would yield

long-chain dimethyl acetals which would appear in the gas chromatograms. No such compounds were detected, so that plasmalogens could have been present only in trace amounts.

PHYTOPLANKTON

The lipids from the phytoplankton sample were fractionated on silicic acid with the following solvents:

Benzene–hexane (15:85, v/v) eluted orange pigments and presumably phytosterol esters and hydrocarbons. This material, 6.7% of the total lipids, was not further investigated.

Hexane–ether (93:7, v/v) eluted triglycerides, free fatty acids and a trace of yellow pigments (34% of the total lipids). After removal of the free fatty acids on aluminum oxide, the triglycerides were treated with pancreatic lipase. Table II gives the composition of fatty acids in the α- and β-positions.

TABLE II. Fatty acid composition of lipids from a diatom, *Skeletonema costatum*, in mole per cent.[a]

		Fatty acids									
	Position	14:0	14:1	16:0	16:1	16:2	16:3	16:4	18:1	18:4	20:5
Triglyceride	α	29	5	13	26	6	4	3	1	<1	5
	β	9	3	2	21	15	11	9	<1	3	20
Galacto-lipids		13	2	3	14	12	14	10	<1	7	21
Lecithin		33	2	4	11	2	4	4	3	17	16

[a]Fatty acids present in less than 2% are omitted.

Ether eluted green pigments and presumably phytosterols and partial glycerides (21% of the total lipids). After deacylation (Brockerhoff, 1963) and separation by two-dimensional paper chromatography (Wintermans, 1960) only one spot was detected which was not identified.

Chlororlorm–methanol (6:1, v/v) eluted green pigments and galactolipids. This fraction, 24% of the total lipids, was also deacylated and separated by two-dimensional paper chromátography (Wintermans, 1960). The mobilities of the two major components corresponded to those of mono- and digalactolipids. There were other components present which were not identified. Table II gives the fatty acid composition.

Three further fractions (14% of the total lipids), eluted with chloroform–methanol 4:1, chloroform–methanol 2:3, and methanol were combined. After deacylation, the components were separated by two-dimensional paper chromatography and located with phosphomolybdate spray (Dawson, 1960). Lecithin, identified as glycerylphosphorylcholine, was the major component. The lecithin (9% of the total lipid) was separated from other components on silicic acid thin-layer plates. The spots corresponding to egg lecithin were eluted with methanol, and the fatty acid composition was determined (Table II).

The phytoplankton, on a wet basis, contained 1.16 g or 2.7% crude lipid with 67% fatty acid (Ackman *et al.*, 1964), of which the triglyceride fraction contained 34%; 15% was present as free fatty acids (probably artifacts), 11% in the partial glyceride fraction and 12.4% in the galactolipid fraction.

DISCUSSION

The most abundant polyunsaturated marine fatty acids are the eicosapentaenoic, 20:5ω3, and the docosahexaenoic, 22:6ω3, acids. These acids are accumulated in the β-positions of triglycerides of fish (Brockerhoff and Hoyle, 1963) and lobster and scallop (Brockerhoff *et al.*, 1963). Taken together with the demonstration of the retention of structure in the digestion and deposition of triglycerides by fish and lobster, the results reported here (Tables I and II) substantiate our hypothesis that the typical fatty acid distribution pattern of triglycerides originates in plankton and is then retained through the marine food chains, until it is broken down in the marine mammals (Brockerhoff and Hoyle, 1963; Brockerhoff *et al.*, 1964). The distribution of the eicosapentaenoic acid in the triglyceride of *Skeletonema* shows that the typical pattern already exists in phytoplankton. Although only one species was investigated, it should be noted that it is a very abundant one: Together with *Thalassiosira*, *Skeletonema* may constitute up to 90% of the spring phytoplankton of the North Atlantic shelf (Fraser, 1962). *Skeletonema* shows a "typical" pattern also in regard to the C_{16} and C_{18} polyunsaturated fatty acids. These acids, however, do not seem to play an important role in the fats of marine animals.

Although support was obtained for the view that the β-monoglyceride structure is retained through the marine food chains, it should be pointed out that this may not be the only factor responsible for the typical fatty acid pattern of marine fats. The retention is not complete (Brockerhoff *et al.*, 1964), and it may be speculated that the still obscure mechanism by which the typical pattern of mammalian fats is established may also operate in fish and in invertebrates. According to a recent plausible proposal (Lands and Hart, 1964) the lecithins may be the agents of fatty acid rearrangement in triglycerides by virtue of the reversibility of their biosynthesis from diglycerides and CDP-choline. In this context, the relatively high content and the typical structure of the lecithin in the zooplankton sample analyzed in the present study should be noted. Little is known about the immediate fate of fatty acids ingested as phosphatides; it is seldom realized that the principal fat of a natural diet must often be a phosphatide, and in particular lecithin, rather than triglyceride.

Pending further investigation, the retention of the triglyceride structure, or retention of the β-monoglyceride bond, may be assumed to operate in terrestrial invertebrates as well as in those of the sea. Triglyceride structures, then, are conserved by invertebrates; they are broken down completely by mammals. It would be in the interest of comparative biochemistry to obtain data on amphibia, reptiles, and birds.

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REFERENCES

ACKMAN, R. G., AND R. D. BURGHER. 1963. Component fatty acids of the milk of the grey (Atlantic) seal. *Canadian J. Biochem. Physiol.*, **41**: 2501–2505.

ACKMAN, R. G., P. M. JANGAARD, R. J. HOYLE AND H. BROCKERHOFF. 1964. Origin of marine fatty acids. I. Analyses of the fatty acids produced by the diatom *Skeletonema costatum*. *J. Fish. Res. Bd. Canada*, **21**: 747–756.

BARRON, E. J., AND D. J. HANAHAN. 1958. Observations on the silicic acid chromatography of the neutral lipids of rat liver, beef liver, and yeast. *J. Biol. Chem.*, **231**: 493–503.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.*, **37**: 911–917.

BROCKERHOFF, H. 1963. Breakdown of phospholipids in mild alkaline hydrolysis. *J. Lipid Res.*, **4**: 96–99.

BROCKERHOFF, H., R. G. ACKMAN AND R. J. HOYLE. 1963. Specific distribution of fatty acids in marine lipids. *Arch. Biochem. Biophys.*, **100**: 9–12.

BROCKERHOFF, H., AND R. J. HOYLE. 1963. On the structure of the depot fats of marine fish and mammals. *Arch. Biochem. Biophys.*, **102**: 452–455.

BROCKERHOFF, H., R. J. HOYLE AND K. RONALD. 1964. Retention of the fatty acid distribution pattern of a dietary triglycerides in animals. *J. Biol. Chem.*, **239**: 735–739.

DAWSON, R. M. C. 1960. A hydrolytic procedure for the identification of individual phospholipids in biological samples. *Biochem. J.*, **75**: 45–53.

FRASER, J. 1962. Geographic and seasonal distribution, Chap. 8, p. 113. *In* Nature adrift: The story of marine plankton. G. T. Foulis & Co., Ltd., London, England.

HANAHAN, D. J., H. BROCKERHOFF AND E. J. BARRON. 1960. The site of attack of phospholipase (Lecithinase) A on lecithin: A re-evaluation. *J. Biol. Chem.*, **235**: 1917–1923.

HANAHAN, D. J., J. C. DITTMER AND E. WARASHINA. 1957. A column chromatographic separation of phospholipids. *J. Biol. Chem.*, **228**: 685–700.

HORNING, E. C., E. H. AHRENS, JR., S. R. LIPSKI, F. H. MATSON, J. F. MEAD, D. A. TURNER AND W. H. GOLDWATER. 1964. Quantitative analysis of fatty acids by gas-liquid chromatography. *J. Lipid Res.*, **5**: 20–27.

KENNEDY, E. P. 1957. Metabolism of lipids. *Ann. Rev. Biochem.*, **26**: 119–148.

LANDS, W. E. M., AND P. HART. 1964. Metabolism of glycerolipids: V. Metabolism of phosphatidic acid. *J. Lipid Res.*, **5**: 81–87.

MATTSON, F. H., AND R. A. VOLPENHEIN. 1962. The use of pancreatic lipase for determining the distribution of fatty acids in partial and complete glycerides. *J. Lipid Res.*, **2**: 58–62.

METCALFE, L. D., AND A. A. SCHMITZ. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.*, **33**: 363–364.

SMITH, I. 1960. Sugars, Chap. 13 Vol. I, p. 248. *In* Chromatographic and Electrophoretic Techniques. Interscience Publishers, Inc., New York.

TREVALYAN, W. E., D. P. PROCTER AND J. S. HARRISON. 1950. Detection of sugars on paper chromatograms. *Nature*, **166**: 444–445.

VOGEL, W. C., W. M. DOIZAKI AND L. ZIEVE. 1962. Rapid thin-layer chromatographic separation of phospholipids and neutral lipids of serum. *J. Lipid Res.*, **3**: 138–140.

WINTERMANS, J. F. G. M. 1960. Concentrations of phosphatides and glycolipids in leaves and chlorophasts. *Biochem. Biophys. Acta*, **44**: 49–54.

Redd Superimposition and Egg Capacity of Pink Salmon Spawning Beds[1]

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ABSTRACT

A study of egg recruitment to pink salmon spawning beds in two southeastern Alaska streams (Indian Creek and Harris River) has shown that egg loss during spawning increases as the density of female spawners increases. Mortality was caused for the most part by superimposition of redds.

A mathematical model of spawning success is derived. The model assumes that the curve describing the recruitment of eggs to a spawning bed is asymptotic and that pink salmon females spawning within any defined spawning ground select the sites of their redds at random. The model is used to estimate the asymptotic limit of the egg recruitment curve for a Harris River spawning bed. Levels of mortality associated with various densities of spawning female pink salmon are predicted for Harris River from the model.

INTRODUCTION

There is evidence that the capacity of a spawning bed to produce fry is impaired by excessive numbers of adults spawning. Ricker (1954, 1958), Neave (1958), and Wickett (1958, 1962) reported that the average yield of fry approached an upper limit as the density of spawners approached an "optimum level". Ricker concluded that maximum fry yield would most likely be realized by allowing spawners to attain a density intermediate between low and high (curve A, Fig. 1). A mathematical model describing an asymptotic relation (curve B, Fig. 1) between egg deposition and recruitment of juvenile fishes has been developed by Beverton and Holt (1957).

Important objectives of research on pink salmon (*Oncorhynchus gorbuscha*) are to understand factors that place an upper limit on the potential of spawning beds to produce fry and to determine the optimum density of adults that should be allowed to spawn. This paper considers one problem pertaining to these questions—mortality from redd superimposition during spawning.

Redd superimposition becomes an important mortality factor for Pacific salmons at high spawning densities (Gilbert and Rich, 1937; Smirnov, 1947; Morgan and Henry, 1959). In one instance, mortality of pink salmon eggs was estimated to be near 90% at high spawning densities (Semko, 1954). The question of competition for spawning space between pink and sockeye (*O. nerka*) salmon in the Karluk River system, Alaska, was considered by Rounsefell (1958), who concluded that large escapements of pink salmon caused a significant reduction in the reproductive potential of sockeye salmon because of crowding on the spawning beds.

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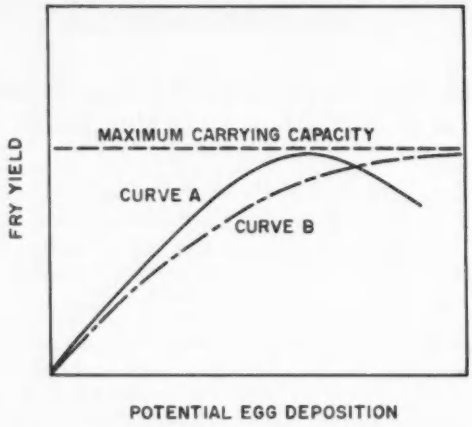

FIG. 1. Postulated relation between potential egg deposition and fry yield of pink salmon. See text for discussion of curves.

The number of fry produced from a spawning bed cannot exceed the number of eggs present at the end of spawning; hence, redd superimposition might place an ultimate upper limit on the potential of spawning beds to produce fry. It is postulated that egg displacement from the spawning bed increases as the density of spawning females increases, ultimately reaching a point where females displace from the spawning bed a number of eggs equal to the number they deposit. In this paper, evidence showing a direct relation between egg loss and density of females spawning is presented; and a mathematical model of spawning success is developed. The model is used to estimate the egg capacity of a pink salmon spawning bed in a southeastern Alaska stream and to predict mortality from redd superimposition.

FIELD STUDY METHODS

The success of pink salmon spawning was evaluated in two southeastern Alaska streams—Harris River and Indian Creek—which are located on Prince of Wales Island about 40 miles west of Ketchikan. Observations were made on intertidal spawning beds utilized almost exclusively by pink salmon. The Harris River study area encompassed 6093 m^2 and the Indian Creek study area 3905 m^2.

A measure of spawning success was obtained from observed differences between two population estimates—(1) the number of pink salmon females estimated to spawn in a study area, and (2) the equivalent number of females required to deposit the number of eggs estimated to be present in the study area after spawning. Methods used to obtain these estimates will be described.

ESTIMATING NUMBER OF FEMALES SPAWNING

The number of pink salmon females spawning in each study area was estimated by counting daily those that were digging and protecting redds and by

determining from tagging studies the average length of time a female remained on the spawning ground. The method is illustrated with pink salmon spawning in Harris River in 1960.

The number of pink salmon females counted daily in the Harris River study area is shown by an eye-fitted curve in Fig. 2. The area under the curve represents 31,700 female days. To obtain an estimate of the average duration a pink salmon female occupied the spawning ground, 38 tagged pink salmon

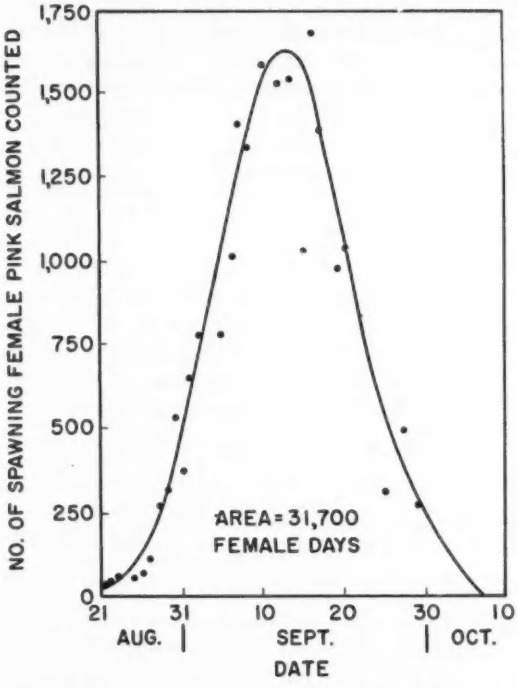

FIG. 2. Daily counts of female pink salmon spawning in the Harris River study area in 1960.

females were observed daily. This gave an average of 9.8 days. One day was added to the total because it was assumed that the average female was present one-half day before the time of the first observation and one-half day after the time of the last. The number of females spawning in the Harris River study area was estimated to be

$$\frac{31{,}700 \text{ female days}}{10.8 \text{ days}} = 2930 \text{ females.}$$

Similar estimates were made for other brood years in Harris River and for Indian Creek. The method and its assumptions are described in more detail by McNeil (1962).

Estimating Equivalent Number of Females Spawning Safely

The abundance of eggs in each study area was estimated by sampling with a hydraulic sampler after spawning (McNeil, 1962). An equivalent number of females spawning safely (i.e. without having their eggs subsequently dislodged) was calculated by dividing the estimated number of eggs in a study area by the assumed average fecundity of pink salmon[2].

Differences between the estimated number of females spawning and the equivalent number spawning safely were thought to be a measure of voided eggs lost during spawning, because very few eggs were found in body cavities of the spent females examined. At low spawning density, there was good agreement between the expected and observed density of eggs in spawning beds; and with the possible exception of 1961, losses at higher spawning densities were attributed mostly to redd superimposition. Flooding occurred before postspawning sampling in 1961 and may have accounted for much of an observed high egg loss.

SPAWNING SUCCESS

A major purpose of this study was to develop analytical methods for predicting egg capacity of spawning beds from estimates of spawning success. The methods described are based on two assumptions: (1) spawning success is limited primarily by redd superimposition, and (2) egg capacity approaches an asymptotic limit with increasing density of spawners. Results of field studies in Indian Creek and Harris River support these assumptions.

Field Observation of Spawning Success

Pink salmon usually spawn in Indian Creek and Harris River during September, and sampling to estimate egg abundance was done between late September and early November after the end of spawning. Density of female pink

[2]Average fecundity was assumed to be 1700 eggs per female (see Rounsefell, 1957).

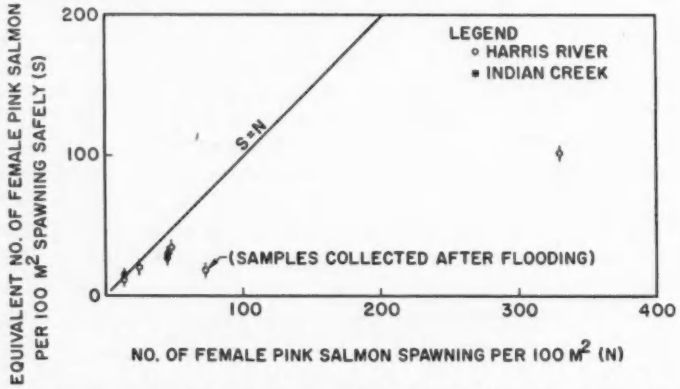

Fig. 3. Observed relation between number of female pink salmon spawning and equivalent number spawning safely. Lengths of vertical bars correspond to 90% confidence interval estimates of population mean.

salmon spawning in the Indian Creek and Harris River study areas and the equivalent number of females estimated to have spawned safely are given in Table I. It is noteworthy that the estimated equivalent number of females spawning safely (S) was consistently less than the estimated total number of females spawning (N), where $N > 24$ females per 100 m^2. Values of S are plotted against their corresponding values of N in Fig. 3 to illustrate the observed relation between these parameters.

TABLE I. Estimated number of female pink salmon spawning and equivalent number required to spawn safely to deposit the number of eggs estimated to be present in the study areas after spawning, Harris River and Indian Creek.

Study area and year	Estimated number of females spawning per 100 m^2 (N)	Estimated equivalent number spawning safely per 100 m^2 (S)	
		Mean	90% confidence limits of mean
Indian Creek			
1958	12.9	15.5	±6.7
1959	46.3	26.9[a]	±7.6
1960	46.3	28.6	±8.6
Harris River			
1958	12.9	12.4	±6.0
1959	24.8	19.6[a]	±4.6
1960	48.4	35.1	±7.8
1961	73.0	18.2[a b]	±6.5
1962	330.0	101.7	±15.9

[a]Samples collected on two dates and having mean values not differing significantly were pooled to obtain a single estimate.
[b]Samples were collected after flooding.

Model of Spawning Success

The model assumes that the equivalent number of females spawning safely approaches an asymptote as the density of spawners increases. Figure 4 illustrates the hypothetical relation between total spawners (N) and the equivalent number laying their eggs safely (S). The asymptotic limit of the equivalent number of spawners that can deposit their eggs safely is designated as "L".

To assist in understanding the model and its assumptions, we will first consider a spawning bed to be represented by a "checkerboard" pattern. Each square of the checkerboard will be equivalent to the average area excavated by a female. The number of squares will equal the number of such sites available for spawning (L), and each female is assumed to select a square at random, i.e. independent of whether or not spawning had previously occurred at the site. Squares receiving more than one female will retain, on the average, a number of eggs equivalent to that deposited by one female.

In a natural spawning bed redds lack the symmetry described by the checkerboard model, and also, redds would in most instances be partially rather than

totally superimposed. We will assume that the frequency of occurrence of partially superimposed redds is proportional to the degree of superimposition. Thus the number of eggs lost from superimposition would be equivalent to that of the checkerboard model, and the simplifying assumption that spawning sites are arranged in checkerboard pattern would not affect the model.

The most questionable assumption is that of random distribution of females. Because female pink salmon establish and defend territories during spawning, the assumption that occupation of positions by earlier spawners does not influence the choice of spawning sites by later spawners might not be strictly correct. Questions requiring further investigation include: (1) How long does a female effectively defend her territory? (2) How specific is the location of her territory in relation to the points at which she deposited her eggs? (3) What influence does increasing spawning density have on territorial behaviour?

The equation used to describe spawning success is:

$$S = L(1-e^{-N/L}) \qquad \ldots (1)$$

Equation 1 can be derived by two methods—from a differential equation and from the Poisson distribution.

DERIVATION FROM A DIFFERENTIAL EQUATION. With each small increment in N (ΔN) there will occur a small increment in S (ΔS) (see Fig. 4). The increments ΔS will decrease in size as N increases and ΔN is held constant. The size

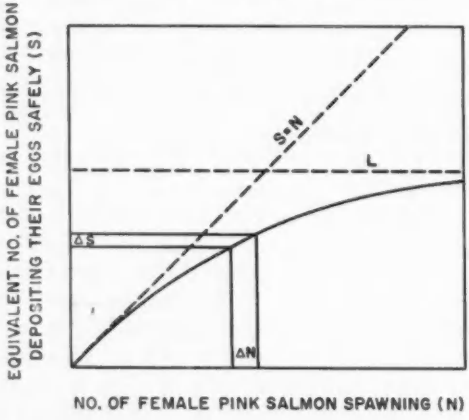

FIG. 4. Relation between number of females spawning in a given area and the equivalent number depositing their eggs safely. ΔS is an increment in S associated with an increment in N(ΔN).

of an increment ΔS will be proportional to the probability that a female entering a spawning ground will select a site which had not already been utilized by another female for spawning. Thus, the increment in S is the increment in N

multiplied by the proportion of the area not previously utilized for spawning at the time ΔN occurs, i.e.

$$\Delta S = \frac{(L-S)}{L} \Delta N \quad \ldots (2)$$

Equation 2 is assumed to be independent of time; hence, spawning behaviour is assumed to be constant throughout the spawning period. Dividing by ΔN and taking the limit as $\Delta N \to 0$ gives:

$$\lim_{\Delta N \to 0} \frac{\Delta S}{\Delta N} = \frac{dS}{dN} = \frac{(L-S)}{L} \quad \ldots (3)$$

where the function is continuous, positive, and single valued between $S = 0$ and $S = L$.

Integrating Equation 3 gives:

$$-ln(L-S) = \frac{N}{L} + C \quad \ldots (4)$$

or:

$$L-S = C' (e^{-N/L}) \quad \ldots (5)$$

The value:

$$C' = L \quad \ldots (6)$$

is obtained by letting $N = 0$.

Substitution of Equation 6 in Equation 5 leads directly to Equation 1:

$$S = L(1-e^{-N/L}).$$

Derivation from the Poisson distribution. Let r be the number of females spawning per available site and let λ be the expected mean frequency of females spawning per available site. In this instance, r is assumed to follow the Poisson distribution where the probability of success can be considered to be a continuous, non-negative function of the number of independent trials (Koopman, 1950; Dixon and Massey, 1957). We thus obtain:

$$\text{Prob}(r) = \frac{(\lambda^r)\ (e^{-\lambda})}{r!} \quad \ldots (7)$$

$$\text{Prob}(r=0) = e^{-\lambda} \quad \ldots (8)$$

$$\text{Prob}(r \neq 0) = (1-e^{-\lambda}) \quad \ldots (9)$$

The proportion of sites utilized by at least one female is $(1-e^{-\lambda})$; hence,

$$\frac{S}{L} = (1-e^{-\lambda}) \quad \ldots (10)$$

The expected mean frequency of females spawning per available site is:

$$\lambda = \frac{N}{L} \quad \ldots (11)$$

It follows from Equations 10 and 11 that the number of females depositing their eggs safely (S) is Equation 1:

$$S = L(1-e^{-N/L}).$$

EGG CAPACITY OF SPAWNING BEDS

Equation 1 can be expressed in linear form, viz.:

$$ln(L-S) = -\frac{1}{L}(N) + lnL \quad . \ . \ . \ (12)$$

where $-\frac{1}{L}$ is the slope and lnL is the point of intercept on the ordinate. By obtaining estimates of N and S, a graphical technique can be used to estimate L. The method will be illustrated with data from Harris River (Table I). The 1961 estimates are not included because flooding occurred before S was estimated and may have caused the removal of significant numbers of eggs from the spawning bed.

After a trial value of L ($L \geq$ the largest estimated value of S), Equation 12 is drawn with slope $= -\frac{1}{\hat{L}}$ and y intercept $= ln\hat{L}$. Values of $ln(\hat{L}-S)$ are calculated for each observed S and are plotted against the corresponding values of N. Different values of $\hat{L}$ are tried until the plotted points exhibit the least deviation from the theoretical line (Equation 12). This is the value accepted as the best estimate of L.

For Harris River, the following numbers of females spawning per 100 m² (N) and the numbers spawning safely per 100 m² (S) were used to estimate L:

	N	*S*
1958	12.9	12.4
1959	24.8	19.6
1960	48.4	35.1
1962	330.0	101.7

Figure 5 shows the line obtained from Equation 12 where L = 106.4 females per 100 m². Larger and smaller trial values of L exhibited greater departure from their theoretical lines, so a value L = 106.4 females per 100 m² of spawning ground was accepted for intertidal Harris River.

Provided each female deposits 1700 eggs, the egg capacity of a spawning bed where L = 106.4 females per 100 m² is

$$\frac{(106.4)\ (1700)}{100} = 1809 \text{ eggs m}^2.$$

This is approximately equivalent to the potential deposition of one female pink salmon per square meter.

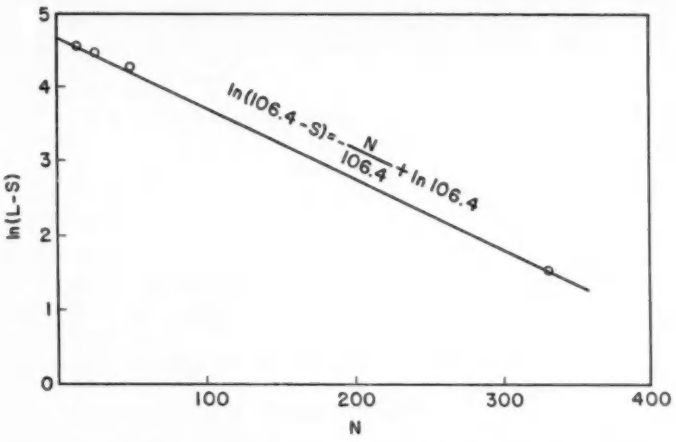

FIG. 5. Line of equation (12) drawn with slope $\left(-\frac{1}{L}\right)$ equal to $-\frac{1}{106.4}$. The plotted points are for Harris River and show the degree of correspondence between the theoretical line and the field observational data.

PREDICTED MORTALITY DURING SPAWNING

If the model adequately describes the dynamics of egg recruitment to the spawning bed and a value of L has been determined with a degree of accuracy commensurate with sampling variations, an estimate of the total mortality percentage (M_t) can be calculated. The number of females required to give any selected number spawning safely can be obtained by solving Equation 12 for N, i.e.

$$N = L(lnL) - L[ln(L-S)] \quad . \ . \ . \ (13)$$

For example, the number of females required to spawn per 100 m² for an equivalent number of 50 to spawn safely (i.e. S = 50), where L = 106.4 is:

$$N = 106.4(ln\ 106.4) - 106.4[ln(106.4-50)] = \frac{68.1 \text{ females}}{100 \text{ m}^2}.$$

The mortality percentage can be calculated from:

$$M_t = \frac{(N-S)}{N}\ (100) \quad . \ . \ . \ (14)$$

and in this instance is found to be 27%. Figure 6 shows how the predicted mortality changes with increasing density of females spawning, where L = 106.4 females per 100 m².

CONCLUSIONS

The development of a successful management program for pink salmon will depend in part on the ability of management biologists to assess fry production potential of spawning beds and optimum escapement of adults to spawning streams. It will be necessary to determine eventually these attributes for each important spawning area and spawning stock.

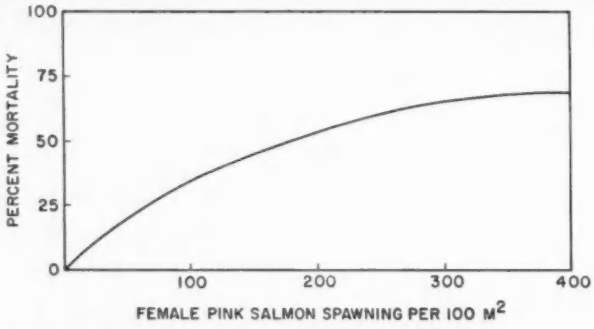

FIG. 6. Predicted relation between density of female pink salmon spawning and egg mortality during spawning where L = 106.4 females per 100 m².

Fry production potential of a spawning bed might ultimately be limited by mortality factors directly related to density of spawning adults. A mathematical model describing the dynamics of mortality from redd superimposition has been advanced in this paper. The model was used to estimate egg capacity of a spawning bed and to predict mortality during spawning. A basic assumption of the model is that females distribute themselves at random over a spawning bed. Further research is required to test the validity of this assumption.

Although redd superimposition is thought to become an important mortality cause at high spawning densities, the potential of a spawning bed to produce fry is possibly limited by additional factors. The oxygen delivery rate, for example, may exert a pronounced influence on carrying capacity. It has been observed, on the other hand, that females remove much extraneous organic matter from spawning beds while excavating redds (Semko, 1954; McNeil and Ahnell, 1964). The removal of eggs through redd superimposition and the cleansing of spawning beds of organic detritus may help the young salmon avoid stresses associated with oxygen privation and help protect them from epidemic outbreaks of pathogenic agents. Redd superimposition may thus be an essential mechanism inhibiting catastrophic mortality due to overcrowding.

In stream areas where spawning occurs over an extended period, mortality from redd superimposition would have its greatest impact on the progeny of early-spawning fish; and in years of large escapement, it is conceivable that late-spawning fish could nearly destroy the progeny of early-spawning fish. The relations between time of spawning and survival potential of young pink salmon are poorly understood. A working hypothesis might be that eggs from a single spawning stock deposited during the middle portion of the run possess a higher survival potential than eggs deposited very early or very late. It is assumed that hereditary differences associated with time of spawning do not exist within the stock. If competition for space on spawning beds becomes critical, as it might in years of great abundance, then the population of embryos surviving at the conclusion of spawning will have originated for the most part from the

adults spawning late in the season. The population might arrive, therefore, at a state where its survival potential is greatly impaired because of the lateness of egg deposition.

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REFERENCES

BEVERTON, R. J. H., AND S. J. HOLT. 1957. On the dynamics of exploited fish populations. *U.K. Min. Agr., Fish. Investig.*, Ser. 2, **19**: 533 pp.

DIXON, W. J., AND F. J. MASSEY, JR. 1957. Introduction to statistical analysis. 2nd edition. McGraw-Hill Book Co., Inc., New York and London. 488 pp.

GILBERT, C. H., AND W. H. RICH. 1927. Investigations concerning the red-salmon runs to the Karluk River, Alaska. *Bull., U.S. Bur. Fish.*, **43**(2): 1–69. (Document No. 1021)

KOOPMAN, B. O. 1950. Necessary and sufficient conditions for Poisson's distribution. *Proc. Am. Math. Soc.*, **1**(6): 813–823.

McNEIL, W. J. 1962. Mortality of pink and chum salmon eggs and larvae in southeast Alaska streams. Ph. D. Thesis, Univ. Washington, Seattle, 270 pp.

McNEIL, W. J., AND W. H. AHNELL. 1964. Success of pink salmon spawning relative to size of spawning bed materials. *U.S. Fish and Wildlife Ser., Special Sci. Rept. — Fish.*, No. 469, 15 pp.

MORGAN, A. R., AND K. A. HENRY. 1959. The 1955–1956 silver salmon run into the Tenmile Lakes System. *Oregon Fish Comm., Research Briefs*, **7**(1): 57–77.

NEAVE, F. 1958. Stream ecology and production of anadromous fish, pp. 43–48. *In* P. A. Larkin, [ed.], The investigations of fish-power problems. H. R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver.

RICKER, W. E. 1954. Stock and recruitment. *J. Fish. Res. Bd. Canada*, **11**(5): 559–623.

1958. Handbook of computations for biological statistics of fish populations. *Bull. Fish. Res. Bd. Canada*, No. 119, 300 pp.

ROUNSEFELL, G. A. 1957. Fecundity of North American Salmonidae. *U.S. Fish. and Wildlife Serv., Fish. Bull.*, **57**: 450–468. (Fish. Bull. No. 123.)

1958. Factors causing decline in sockeye salmon of Karluk River, Alaska. *Ibid.*, **58**: 83–169. (Fish. Bull. No. 130.)

SEMKO, R. S. 1954. [The stocks of West Kamchatka salmon and their commercial utilization.] Zapasy zapadnokamchatskikh lososei i ikh promyslovoe ispolzovanie. *Izv. Tikhookeans. Nauchno-Issled. Inst. Rybn. Khoz. i Okeanogr.*, **41**: 3–109. [Fish. Res. Bd. Canada, Translation Series, No. 288.]

SMIRNOV, A. G. 1947. [Conditions of stocks of the Amur salmon and causes of the fluctuations in their abundance.] Sostoyanie zapasov amurskikh lososei i prichiny ikh chislennykh kolebanii. *Izv. Tikhookeans. Nauchno-Issled. Inst. Rybn. Khoz. i Okeanogr.*, **25**: 33–51. Translation *in* Pacific Salmon, pp. 66–85. Israel Program for Scientific Translations, Jerusalem, 1961.

WICKETT, W. P. 1958. Review of certain environmental factors affecting the production of pink and chum salmon. *J. Fish. Res. Bd. Canada*, **15**(5): 1103–1123.

1962. Environmental variability and reproduction potentials of pink salmon in British Columbia, pp. 73–86. *In* N. J. Wilimovsky, [ed.] Symposium on pink salmon. H. R MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver.

Polyphosphate Treatment of Frozen Cod. 3. Taste Panel Evaluation, Chemical Assessment and Thaw-Drip in Once-Frozen Newfoundland Trap-Caught Cod[1]

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ABSTRACT

Sodium tripolyphosphate treatment before packaging and freezing of fillets of trap-caught cod (*Gadus morhua* L.) *in-rigor* was compared with treatment in plain water.

The once-frozen treated fillets, evaluated by an analytical taste panel after frozen storage at −12°F (−24.5°C) for periods up to 27 weeks, had significantly better texture characteristics than did the untreated control fillets.

Thaw-drip was significantly smaller in treated samples but this effect was less pronounced after extended frozen storage.

Lipid hydrolysis proceeded at the same rate in both treated and untreated fillets.

There was no significant difference between the electrophoretic patterns of the proteins found in the treated and untreated samples. In both cases the muscle albumins constituted the major portion of the proteins in the drip.

Results indicate that polyphosphate-treated fillets prepared from trap cod could have greater market acceptance than untreated fillets. The producer would benefit from improved quality of product and from increased yield.

INTRODUCTION

The purpose of previous work with once-frozen trawler-caught cod (Dyer *et al.*, 1964) and with once- and twice-frozen Newfoundland summer-trap and fall-caught cod (MacCallum *et al.*, 1964; MacCallum, 1964) was to determine differences in extractable protein, lipid hydrolysis, and thaw-drip developed during cold storage of sodium tripolyphosphate-treated and untreated fish. The effect of treatment on yields of thawed fillets was assessed also.

It has been shown that polyphosphates reduce thaw-drip under certain circumstances (Mahon, 1963). In a previous study there was no consistent effect of polyphosphate on thaw-drip for samples previously stored for several weeks in the frozen state (Dyer *et al.*, 1964). To the authors' knowledge there

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is no published information relating polyphosphate treatment with texture and taste characteristics.

Employing an analytical taste panel, the present study has sought to evaluate taste and texture characteristics of treated and untreated fillets during frozen storage, and loss of certain nutrients to the drip.

EXPERIMENTAL

Lightly-feeding and non-feeding cod (*Gadus morhua* L.) of 51–66 cm length were boated at the traps near St. John's on July 29, 1963, the pH taken, and the fish sorted for size and gutted. Males were washed in iced sea water and iced immediately. The temperature of the fish flesh immediately prior to washing was 50–53°F (10–11.7°C). Thirty hours later the *in-rigor* iced fish were again examined and the pH and temperatures of the whole fish and the fillets cut from them were recorded. The two skinned fillets from each fish were weighed separately. One fillet was immediately dipped in one-fourteenth part of its weight of plain tap water, then drained for 30 sec while that from the other side of the fish was dipped in an equal amount of 10.7% solution of sodium tripolyphosphate and drained. Half the water or solution measured for each fillet was poured into the bottom of an enamelled tray; the fillet was laid in the tray and the remaining quantity of liquid was poured over the fillet. The fillet was turned over and all parts of its surfaces introduced to the liquid for a period of about 2 min, at which time liquid uptake appeared to be complete. The process was repeated for 18 fish. Paired fillets of fish of 55–59 cm length (average 57 cm) were individually packed in 1-lb waxed paper cartons, overwrapped in Saran, sealed and frozen for taste panel assessment. Fillets of fish of 51–66 cm length (average 59 cm) were likewise packed and frozen for physical and chemical analyses. The packages were frozen in 1¼ hr in a horizontal-plate freezer in the Job Brothers and Company Southside Plant, St. John's, Newfoundland. The individual cartons were placed in a sealed, insulated plywood box and the whole placed in cold storage. The air within the box was at a temperature of −12°F (−24.5°C) or lower throughout the storage period except for two instances when the temperature rose to −10°F (−23°C) and quickly returned to −12°F.

Taste panel evaluation of samples followed the procedures of Dyer and Dyer (1949) modified for frozen fish. Samples were baked, examined for texture, taste, and odour and scored principally on the basis of the first two qualities with more weight given to texture. Texture, taste, and "overall acceptability" scores are reported on a scale of 0–100; 40 represents the dividing line between acceptable and unacceptable samples, and 80–90 denotes best quality fish. Physical and chemical analyses followed the procedures listed: salt extractable protein (EPN), Dyer *et al.* (1950, 1964); total lipid (TL), Bligh and Dyer (1959); free fatty acids (FFA) were titrated against alcoholic NaOH, and results calculated for a fatty acid average molecular weight of 282; drip as percentage of wet weight, Dyer *et al.* (1962); phosphorus, colorimetric determination following the method of Harris and Popat (1954); total and non-protein nitrogen (NPN) in the drip by the procedure of Campbell and Hanna (1937) modified; vertical starch gel

electrophoresis of the drip proteins by the method of Smithies (1959). Frozen samples of the drip were shipped in a dry ice refrigerated container to the Technological Research Laboratory, Halifax, Nova Scotia, for vertical starch gel electrophoresis analysis.

RESULTS AND DISCUSSION

Quantities Sampled

At each test period, three cartons of treated and three of untreated fish were used in the taste tests. Three other cartons of each type were used for chemical and physical tests.

Phosphorus Uptake

Phosphorus uptake (Table I) in treated samples amounted to 0.14% P (2 weeks storage), 0.08% P (3 months storage), and 0.14% P (6 months storage). Expressed as sodium tripolyphosphate (25% P) this represents uptakes of 0.56, 0.32, and 0.56%, respectively, or an average of 0.48%. Had all the tripolyphosphate used been absorbed uniformly throughout the flesh, maximum average uptake would have been 0.77%. Total phosphorus in untreated controls (Table I) averaged 0.20%, in agreement with previously reported values in cod flesh (Dyer *et al.*, 1958; MacCallum *et al.*, 1964).

Table I. Physical and chemical data on sodium tripolyphosphate and untreated frozen, stored fillets of trap-caught cod.

Sample	Weeks in frozen storage	pH	Percentage wet weight[a]				FFA percentage total lipid[a]
			Drip	P	TL	EPN	
A[a]	2	6.50	3.4	0.33	0.84	1.90	17.2
B[b]	2	6.42	10.5	0.19	0.86	1.91	14.4
A	14	6.32	6.3	0.27	0.92	2.04	21.2
B	14	6.46	8.2	0.19	0.84	2.12	21.4
A	27	6.62	8.1	0.35	0.77	1.83	26.3
B	27	6.49	10.8	0.21	0.88	1.96	24.8

[a]Treated fillets.
[b]Untreated fillets.
[c]Average of three analyses.

Total Lipid and FFA

Total lipid was nearly as high as that reported for 1962 trap fish (MacCallum *et al.*, 1964, and unpublished data) and was not influenced by treatment (Table I). Free fatty acids percentages were in agreement with those reported in the above-mentioned papers and amounts formed were independent of the dip treatment.

EPN

Protein extractability by salt solution (Table I) was similar to that reported for 1962 trap fish (MacCallum *et al.*, 1964, and unpublished data) and was not influenced by pre-freezing treatment of the fillets.

Percentage Drip

Drip from untreated samples (Table I) was about equal to the mean reported for 1962 samples (MacCallum *et al.*, 1964, and unpublished data), despite the fact that 1962 samples were essentially handled "dry" during filleting operations. Treatment with sodium tripolyphosphate resulted in consistently lower drip values. The averages over 27 weeks frozen storage were 5.9% and 9.8%, respectively, for treated and untreated paired fillets, a significant difference. The addition of polyphosphate reduced drip by 40%.

Analyses on Drip

There was no significant difference in percentage protein lost in the drip from treated and untreated fillets (Table II).

At Halifax the frozen drip samples were thawed and a vertical starch gel electrophoresis run was made on each individual sample. The conditions used for the electrophoresis run were those described by Odense and Shinners (1962).

Table II. Analyses on drip from sodium tripolyphosphate treated and untreated frozen, stored fillets of trap-caught cod.

Sample	Weeks fillets in frozen storage	Total nitrogen[a]	NPN[a]	PN[a]	Percentage protein in drip[a]
A[b]	2	12.8	4.1	8.7	5.2
B[c]	2	11.8	4.1	7.7	4.8
A	6	13.1	4.3	8.5	5.5
B	6	12.9	4.2	8.6	5.4
A	14	12.3	4.2	8.1	5.1
B	14	11.2	4.2	7.0	4.4
A	27	11.1	3.9	7.2	4.5
B	27	11.3	4.1	7.6	4.8

[a]Average of three analyses expressed in milligrams/millilitre.
[b]Sample from treated fillets.
[c]Sample from untreated fillets.

Figure 1 is a photograph of two gels. Gel A is the electrophoresis run of some samples of a water extract of cod muscle containing the muscle albumin proteins. Gel B is the electrophoresis run of 6 drip samples after 14 weeks storage. Numbering from left to right the samples 1, 2, 4, 7, 8 and 10 are controls and 3, 5, 6 and 9 are treated samples. The samples 1 and 6, 2 and 7, 3 and 9, and 4 and 10 are duplicates. The following observations can be made.

1. There is no significant difference between the patterns of the treated and the untreated drip samples.

2. There is a higher protein concentration in the drip sample than in the water extract of the muscle albumins. This is to be expected since the drip is an undiluted exudate from the muscle.

3. The electrophoretic pattern of the drip closely resembles the banding pattern of the muscle albumins. However, there is a considerable variation in

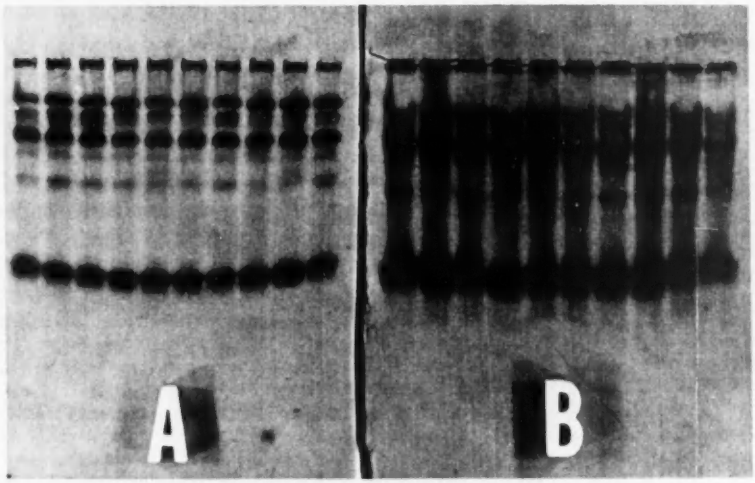

FIG. 1. Starch gel electrophoresis patterns of cod muscle albumins (gel A) and drip samples (gel B). Cathode was at top end of gel.

the relative amounts of the proteins in the different bands. It may be concluded that the drip proteins are principally muscle albumins but their relative concentrations differ considerably from those found in a water extract of muscle albumins.

A visual comparison of the intensity of the staining revealed that the protein concentration of the drip samples increased from 2 to 14 weeks in storage but started to decrease again by the 27th week in storage.

TASTE PANEL

Grades given untreated fillets were lower than those for once-frozen fillets of St. John's trap fish caught in 1962 (MacCallum *et al.*, unpublished data). However, texture and overall grades were improved significantly as a result of dipping

TABLE III. Taste panel evaluation of sodium tripolyphosphate treated and untreated frozen, stored fillets of trap-caught cod.

Sample	Weeks in frozen storage	Texture	S.E.	Taste	S.E.	Overall acceptability	S.E.	N
A[a]	1	72	±1.9	64	±3.6	70	±3.6	22
B[b]	1	51	±5.8	63	±3.3	58	±4.0	23
A	14	63	±4.9	60	±4.5	64	±3.7	24
B	14	36	±4.3	69	±3.9	50	±3.7	23
A	27	63	±3.2	60	±2.5	64	±2.9	24
B	27	50	±4.3	61	±4.0	54	±3.1	24

[a]Treated fillets.
[b]Untreated fillets.

the fillets (Table III). Treated samples tasted 1 week after freezing were pleasantly soft to only slightly fibrous whereas untreated fillets were medium to very chewy. At 14 weeks untreated samples were chewy and tough while treated samples were slightly to medium fibrous. After 27 weeks storage treated samples continued to be preferred and had significantly (5% level) softer texture. Previously, drip losses were shown to be reduced sometimes as the result of sodium tripolyphosphate treatment of fillets (MacCallum *et al.*, 1964). The consistent benefit to texture resulting from the dip used with 1963 samples is significant and may be correlated with consistent reduction in drip (see above). The most interesting result from the standpoint of the consumer, however, is the apparent slowing down of texture changes during storage. In regard to taste, only one of the treated samples was slightly sweet; others were not markedly different in taste from untreated samples and taste ratings for treated and untreated fillets were generally similar.

REFERENCES

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.*, **37**: 911–917.

CAMPBELL, W. R., AND M. I. HANNA. 1937. The determination of nitrogen by modified Kjeldahl method. *J. Biol. Chem.*, **119**: 1–7.

DYER, W. J., H. BROCKERHOFF, R. J. HOYLE AND DORIS I. FRASER. 1964. Polyphosphate treatment of frozen cod. I. Protein extractability and lipid hydrolysis. *J. Fish. Res. Bd. Canada*, **21**(1): 101–106.

DYER, F. E., AND W. J. DYER. 1949. Changes in the palatability of cod fillets. *J. Fish. Res. Bd. Canada*, **7**: 449–460.

DYER, W. J., DORIS I. FRASER AND J. R. DINGLE. 1958. Sodium phosphate crystals on saltfish. *Nature*, **181**: 858–859.

DYER, W. J., DORIS I. FRASER, D. G. ELLIS, D. R. IDLER, W. A. MACCALLUM AND E. J. LAISHLEY. 1962. Quality changes in stored refrozen cod fillets. *Supplement au Bulletin de l'Institut International du Froid, Annexe* 1962–1, pp. 515–524.

DYER, W. J., H. V. FRENCH AND J. M. SNOW. 1950. Proteins in fish muscle. I. Extraction of protein fractions in fresh fish. *J. Fish. Res. Bd. Canada*, **7**: 585–593.

HARRIS, W. D., AND PRANJIVAN POPAT. 1954. Determination of the phosphorus content of lipids. *J. Am. Oil Chemists' Soc.*, Vol. 31, pp. 124–127.

MACCALLUM, W. A. 1964. Temperatures and thaw-drip associated with electronic thawing of Newfoundland cod. *J. Fish. Res. Bd. Canada*, **21**: 719–726

MACCALLUM, W. A., H. S. SHIEH, DOROTHY A. CHALKER, W. J. DYER AND D. R. IDLER. 1964. Polyphosphate treatment of frozen cod. 2. Effect on drip, yield, lipid hydrolysis and protein extractability in twice-frozen Newfoundland summer trap and fall cod. *J. Fish. Res. Bd. Canada*, **21**(3): 539–548.

MAHON, J. H. 1962. U.S. patent 3,036,923.

ODENSE, P. H., AND C. W. SHINNERS. 1962. Direct vertical starch gel electrophoresis of cod muscle tissue. *Canadian J. Biochem. Physiol.*, **40**: 1840–1843.

SMITHIES, O. 1959. An improved procedure for starch-gel electrophoresis. *Biochem. J.*, **71**, 585.

Growth, Reproduction, and Distribution of Pandalid Shrimps in British Columbia[1]

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ABSTRACT

The ranges, habitats, and commercial importance are described for nine species of the family Pandalidae: *Pandalopsis dispar, Pandalus jordani, P. borealis, P. platyceros, P. hypsinotus, P. danae, P. montagui tridens, P. stenolepis, P. goniurus.* A key for their identification is given. Over 27,400 specimens were sampled, using trawl and trap gear, mostly from the Strait of Georgia. *Pandalopsis dispar* is a male at 1½ years, attaining a total length of 136 mm and body weight of 6.5 g; at 30 months, in the female phase, the length is 169 mm and weight 16.3 g. *Pandalus jordani* matures as a male, and to a lesser extent a female, at about 18 months, reaching a length of 105 mm and weight of 3.8 g; at 2½ years all are in the female phase, averaging 118 mm in total length and 5.8 g in body weight. *P. platyceros* is first a male at 1½ years, attaining a length of 140 mm and weight of 14.2 g; most remain as males for another year, and at 3½ years the total length is 180 mm and weight 35.5 g. *Pandalus hypsinotus* matures as a male, and to some extent as a female, at about 1½ years, at a total length of 124 mm and weight of 10.3 g; at 30 months the length is 130 mm and weight 12.7 g. Almost as many females as males of *P. borealis* mature at 1½ years, attaining a length of 95 mm and weight of 3.2 g; at 30 months, in the female phase, the length is 119 mm and weight 6.1 g. *Pandalus danae* matures first as a male, and to some extent as a female, at 1½ years, reaching 88 mm in length and 4.4 g; in the female phase, at 2½ years the length is 105 mm and weight 8.5 g. Growth of *P. montagui tridens, P. stenolepis, and P. goniurus* is uncertain, but all undergo sex change. Growth as related to habitat and early maturing of females as related to geographic and bathymetric ranges are discussed.

INTRODUCTION

Nine species of shrimps, belonging to two genera, *Pandalus* and *Pandalopsis*, of the family Pandalidae (order Decapoda) are found off the coast of British Columbia. All occur in commercial catches at some time or other but three species, *Pandalus goniurus* Stimpson, *P. stenolepis* Rathbun, and P. *montagui tridens* Rathbun, are scarce and generally remain unrecognized by fishermen. In recent years the province's total annual catch of shrimps has averaged 1.4 million lb, with a landed value of about $230,000. In addition to their economic importance, pandalid shrimps are of great interest biologically. With but one or two known exceptions, all species investigated exhibit protandric hermaphroditism.

The geographic and bathymetric distributions of Pandalidae of the west coast of North America were described by Rathbun (1904). In two short papers, Berkeley (1929a, b) reported sex change in *Pandalus danae* Stimpson and four other species. Hynes (1929) in his description of the shrimp fishery near Peters-

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burg, Alaska, tabulated average lengths and egg counts of ovigerous females of six species. The classic work of Berkeley (1930) described the larval stages, and subsequent growth and reproduction of five commercial species from British Columbia. In his report on the status of the fishery in Puget Sound, Smith (1937) measured samples of *Pandalus danae*, *P. goniurus*, and *P. jordani*, and determined associated fishes and invertebrates. The larval development of another species, *P. stenolepis*, was described by Needler (1938). On the basis of sampling conducted off Oregon, Pruter and Harry (1952) concluded that *Pandalus jordani* underwent sex change during a life cycle similar to *P. borealis*. Tegelberg and Smith (1957) determined sex and age composition of *P. jordani*, and its ovigerous period from collections off the Washington coast. Three well-defined size groups occurred in samples of *P. jordani* caught during exploratory fishing off central Oregon, but Ronholt and Magill (1961) did not attempt to assign ages to these. During the decade ending in 1960, exploration for new shrimp fishing grounds was conducted extensively from Alaska to California; these operations are summarized by Greenwood (1959) and Alverson *et al.* (1960).

Several of the above-mentioned species have been studied in the Asian and European sectors of their ranges. Igarashi (1951) investigated growth and reproduction of *Pandalus hypsinotus* Brandt in Japanese waters. About a dozen accounts of the biology of *P. borealis* have been written; those of Hjort and Ruud (1938), Rasmussen (1953), and Allen (1959) are mentioned here. Mistakidis (1957) carried out a detailed study of the European form of *P. montagui*.

The present paper is a further contribution to the knowledge of the biology of pandalid species originally studied by Alfreda Berkeley Needler, extended to include species not studied by her. This investigation was concerned mainly with the growth and age, sex change, and distribution in the early post-larval and adult lives of these shrimps.

METHODS

Most of the some 27,400 specimens of nine pandalid species, *Pandalopsis dispar*, *Pandalus jordani*, *P. borealis*, *P. platyceros*, *P. hypsinotus*, *P. danae*, *P. montagui tridens*, *P. stenolepis*, *P. goniurus*, measured and sexed, were obtained by experimental fishing with the Fisheries Research Board of Canada research trawler, *Investigator No. 1*, on grounds in the Strait of Georgia (Fig. 1 and 2). Up to 87 stations, from 10 to 140 fathoms (18–252 m), were fished at bi-monthly intervals between early November 1960 and October 17, 1962. Tows were made with a 40-ft flat shrimp otter trawl (Schaefers and Johnson, 1957) constructed throughout of 1½-inch mesh (knot to knot) cotton netting with a panel of ½-inch mesh light cotton net lining the last 2 ft of the cod end. Most tows lasted 15 min. Traps used were rectangular, 24 × 12 × 12 inches, covered either completely or partly with 1½-inch netting and with a circular opening at each end (Butler, 1963). Traps, baited with frozen herring or dogfish carcasses, were normally left in the water 24 hr.

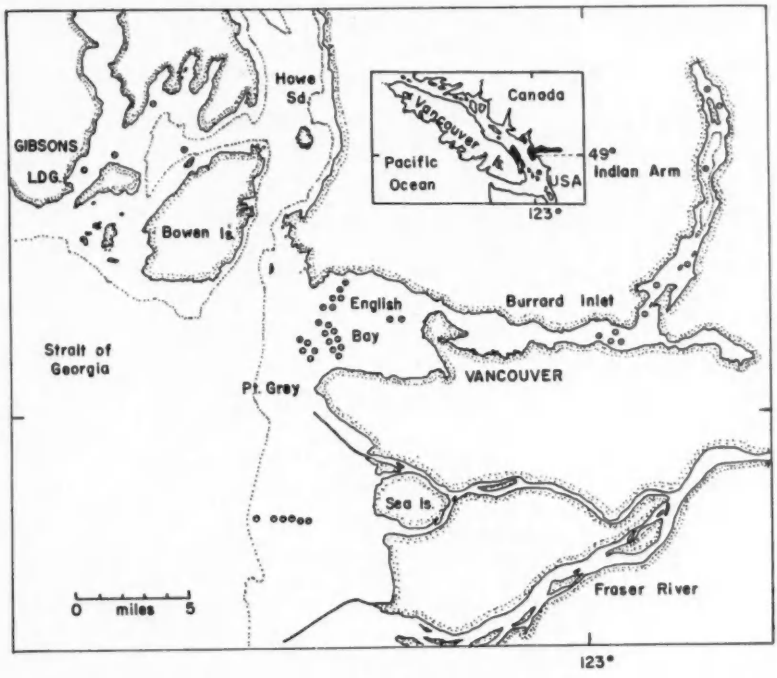


FIG. 1. Map of shrimp trawl tows in Howe Sound, English Bay, and Indian Arm; 100-fathom contour line shown. Inset shows location of sampling regions in Strait of Georgia. Circles represent trawl tows.

In examining each trawl and trap catch, fishes and invertebrates were removed and the shrimps were weighed. Counts of freshly caught shrimps in 1-lb samples were taken and generally 50–100 of the same specimens were sexed and measured at sea. In most cases, examination of the endopodite of the first pleopod sufficed for sex determination but at times it was necessary to observe the state of the appendix masculina, if present, on the endopodite of the second pleopod. Specimens for which the sex was doubtful were preserved for later examination. The carapace length, from the base of the eyestalk to the posterior mid-dorsal edge, was measured with a vernier caliper. Measurements were recorded to the nearest 0.1 mm, and for subsequent graphing were placed in 0.2-mm groups. The means of size groups have been placed on most length frequency diagrams. The relationship between carapace length and total length was determined: conversion factors and equations of regression lines are shown in Table I. Early in October 1962, shrimps were measured and weighed to determine the carapace length–total body weight relationships of six species. After measuring, shrimps (sexes combined) were placed in 0.2-mm groups and each

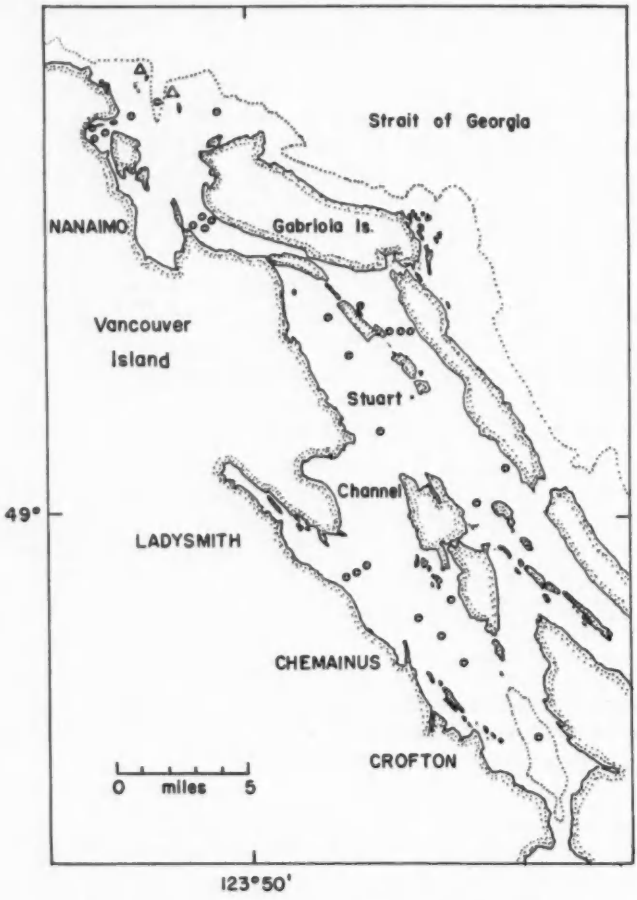


FIG. 2. Map of trawl tows and trap sets along the east coast of Vancouver Island; 100-fathom contour line shown. Circles represent trawl tows, triangles trap sets.

group was weighed to the nearest 0.1 g on a triple beam balance aboard the vessel. The mean weight of shrimps in each group was determined and used with means of carapace lengths to calculate the equations of regression lines shown in Table II. Unless otherwise stated all measurements in the text are of carapace length.

After selected tows, bottom water temperatures were taken with a deep-sea protected reversing thermometer and bathythermograph. Samples for salinity determinations were obtained with a Nansen water bottle.

TABLE I. Conversion factors and equations of regression lines for relationships of carapace length to total length of six pandalid shrimps. Y = total length, tip of rostrum to tip of telson, X = carapace length; both in millimetres.

Species	No. in sample	Sex	Conversion factor	Equation
Sidestripe	278	♂	6.29	Y = 24.08 + 5.10X
(*Pandalopsis dispar*)		♀	5.78	Y = 77.24 + 3.16X
Smooth pink	137	♂	5.96	Y = 6.73 + 5.54X
(*Pandalus jordani*)		♀	5.83	Y = 13.98 + 5.19X
Pink	234	♂	5.93	Y = 1.32 + 5.84X
(*P. borealis*)		♀	5.76	Y = 6.05 + 5.43X
Prawn	247	♂	5.05	Y = −1.65 + 5.10X
(*P. platyceros*)		Trans	5.04	Y = 11.57 + 4.72X
		♀	4.76	Y = 49.86 + 3.62X
Humpback	245	♂	5.14	Y = 6.65 + 4.76X
(*P. hypsinotus*)		♀	5.01	Y = 8.95 + 4.66X
Coonstripe	150	♂	5.07	Y = 7.49 + 4.55X
(*P. danae*)		♀	4.83	Y = 0.96 + 4.74X

TABLE II. Length–weight relationships of six pandalid shrimps. W = total body weight in grams, and L = carapace length in millimetres.

Species	No. in sample	Equation
Sidestripe (*Pandalopsis dispar*)	232	Log W = 3.00750 log L − 3.20020
Smooth pink (*Pandalus jordani*)	353	Log W = 3.17344 log L − 3.37631
Pink (*P. borealis*)	260	Log W = 2.60977 log L − 2.64693
Prawn (*P. platyceros*)	333	Log W = 2.93148 log L − 3.07787
Humpback (*P. hypsinotus*)	199	Log W = 2.78578 log L − 2.83882
Coonstripe (*P. danae*)	317	Log W = 2.92287 log L − 2.97775

IDENTIFICATION OF PANDALID SHRIMPS

The following key, based on one devised by Rathbun (1904), has been of value in the identification of all British Columbia pandalids.

1. Antennules twice the length of the carapace; merus of third maxilliped, and ischium of first pereiopod with longitudinally developed laminate expansions, with long bristles. *Pandalopsis dispar*

Antennules not longer than carapace; no laminate expansions on merus of third maxilliped or ischium of first pereiopod. (2)

2. Third segment of abdomen in part compressed and carinated, the carina forming a more or less well-defined lobe or spine in front of the posterior margin.	(3)
Third segment of abdomen not compressed or carinated.	(5)
3. Third and fourth segments of abdomen each armed with a median spine on the posterior margin.	*Pandalus borealis*
Third and fourth segments of abdomen without median spines.	(4)
4. Rostrum unarmed on distal half of dorsal margin.	*P. goniurus*
Rostrum with spines on distal half.	*P. jordani*
5. Dorsal spines not present behind middle of carapace.	(6)
Dorsal spines present behind middle of carapace.	(7)
6. Sixth abdominal segment more than twice as long as wide.	*P. montagui tridens*
Sixth abdominal segment less than twice as long as wide.	*P. platyceros*
7. Dorsal spines more than 15 (17–21).	*P. hypsinotus*
Dorsal spines less than 15 (8–12).	(8)
8. Antennal scale very narrow, distal half of blade narrower than the thickness of the adjacent spine.	*P. stenolepis*
Antennal scale of moderate width, distal half of blade wider than the thickness of the adjacent spine.	*P. danae*

GROWTH AND REPRODUCTION

Pandalopsis dispar Rathbun

This species occurs from the Bering Sea to the Washington coast, at depths of 25–350 fathoms (45–642 m). It is commonly called the "side-stripe" shrimp because of the white striping on the abdomen. In British Columbia waters, commercial quantities are taken by trawling (40 fathoms and deeper) in English Bay, Barkley Sound, and Chatham Sound.

Hynes (1929) measured, but apparently did not sex, 4500 specimens of *Pandalopsis dispar*; the average total length of all was given as 91 mm. The average egg count for 25 ovigerous specimens was 4150.

Four larval stages were described by Berkeley (1930), and also the growth and reproduction of adults, based on rather scanty material. Most of the larvae hatch in March or April, and the first three stages were found in deep water near the site of hatching. The smallest individuals, males averaging about 60 mm in total length, were found late in August at 120 fathoms (220 m) in Howe Sound. Males matured in the second autumn, at about 18 months, and remained sexually active for another year. Change of sex began in the spring, 3 years after hatching, and in the autumn these individuals, by then females, became ovigerous. Growth of females, then an estimated 4 years, following egg hatching was unknown because very few individuals were found to measure.

In the present study, a total of 6908 specimens of *P. dispar* was measured and sexed. Samples of this species were taken entirely by trawling on soft mud bottoms, between 25 and 141 fathoms (45–154 m).

Figure 3 shows the length composition of 2868 specimens sampled along the east coast of Vancouver Island, 25–110 fathoms, between Nobember 8, 1960,

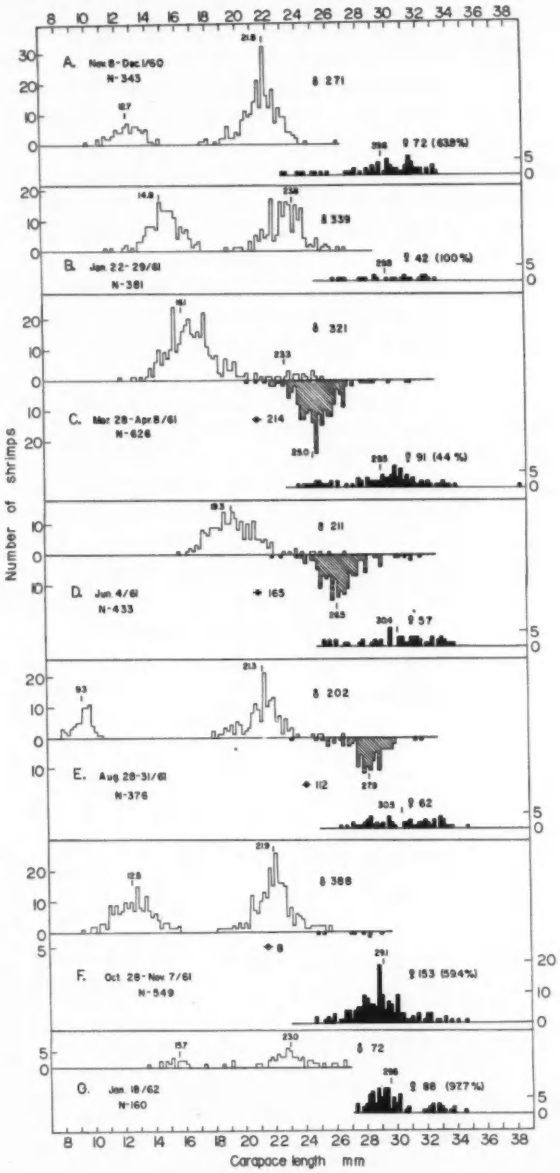


FIG. 3. *Pandalopsis dispar*. Carapace length–frequency distributions from east coast of Vancouver Island, November 8, 1960, to January, 16, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

and January 18, 1962. Spawning was well advanced in the late fall and early winter samples (Fig. 3A, B) as most females then were ovigerous. Eggs were carried throughout the winter (Fig. 3B, G), and were in the process of hatching in late March and early April (Fig. 3C). Larval life then began and lasted for several months. By late August the larvae had metamorphosed and formed the group of small males graphed in Fig. 3E. These males averaged 12.5 mm by late October and early November (Fig. 3F) when they were about 7 months old. This group of males corresponded to the group with a mean of 12.7 mm sampled 11 months earlier (Fig. 3A). The latter group was conspicuous in a later sample (Fig. 3B), and by late March and early April (Fig. 3C), a year after hatching averaged 16.1 mm. Growth of these males was rapid through the summer (Fig. 3D, E), increasing about 1 mm per month. In the sample taken 2 months later (Fig. 3F) these males, then about 19 months old, were mature. Growth of the second-year males continued through the winter (Fig. 3B, G). By late March and early April, the larger males (mean 23.3 mm) were then 2 years old but most individuals in this age group were in the transition stage (Fig. 3C). The changing of sex continued through the summer (Fig. 3D, E), until late autumn (Fig. 3F). The females which had recently changed sex were conspicuous in this latter sample. Their age then was about 31 months. By the time the eggs began to hatch in the spring these females had completed their third year. Some females appear to survive into the fourth year but since size groups are not separated clearly the growth rate is uncertain.

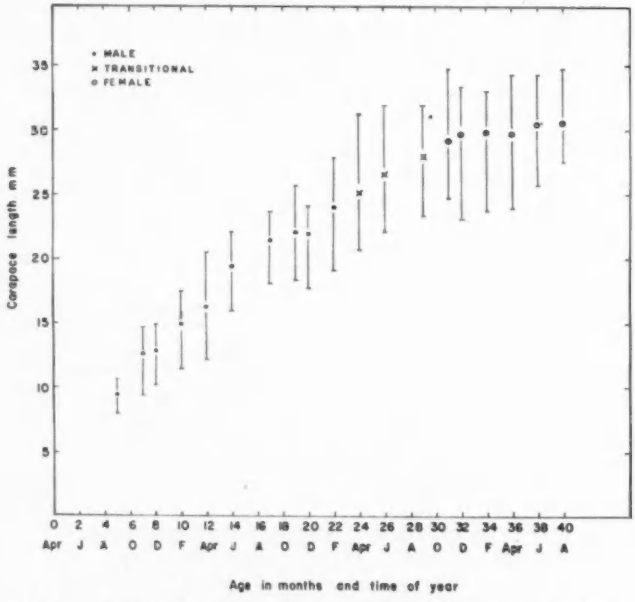

• MALE
× TRANSITIONAL
○ FEMALE
Carapace length m.m
35
30
25
20
15
10
5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Apr J A O D F Apr J A O D F Apr J A O D F Apr J A
Age in months and time of year

FIG. 4. *Pandalopsis dispar*. Means and ranges of carapace lengths according to age, sampled from east coast of Vancouver Island.

The means and ranges of carapace lengths of *P. dispar* of Vancouver Island samples described above are summarized in a growth curve in Fig. 4.

Other samples of *P. dispar* from English Bay, Indian Arm, and Howe Sound are graphed in Fig. 5, 6, and 7, respectively. In general, these distributions are similar to those in Fig. 3, and therefore will not be described in detail. The means of the age groups in these three areas at the same sampling periods are at most 0.5–1.5 mm higher than along the east coast of Vancouver Island; most pronounced are the male groups which appear to have a faster growth rate in English Bay. The smallest transitional individual shown in Fig. 3C was 20.6 mm; this and others less than about 24 mm in subsequent samples (Fig. 3D, E)

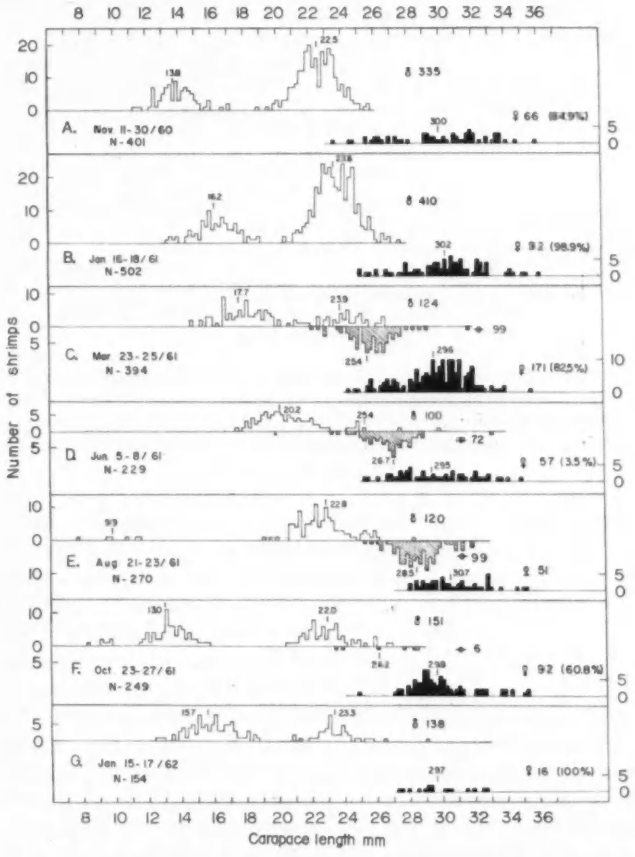


FIG. 5. *Pandalopsis dispar*. Carapace length–frequency distributions from English Bay, November 11, 1960, to January 17, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

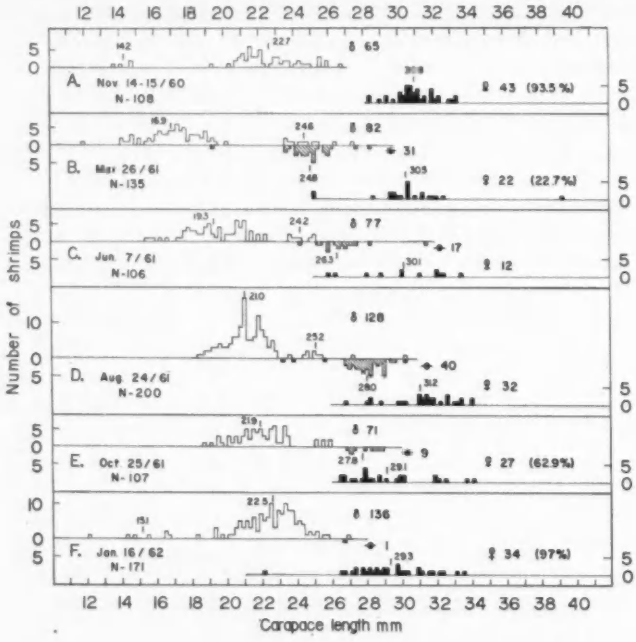


FIG. 6. *Pandalopsis dispar*. Carapace length–frequency distributions from Indian Arm, November 14, 1960, to January 16, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

are considered as slow-growing 2-year-olds. In the sample from Indian Arm on March 26, 1961 (Fig. 6B), one yearling measuring 19.2 mm was transitional. As shown by the fall samples in 2 consecutive years, spawning appears to start around the same time in all areas. However, variations in hatching dates are in evidence; a comparison of the percentages of ovigerous females in the samples from March 23 to April 8, 1961, indicates a sequence of hatching in the Howe Sound, Indian Arm, east coast of Vancouver Island, and English Bay areas. In fact, hatching was so retarded in the last area in 1961, at least, that ovigerous females were present between June 5 and 8 (Fig. 5D). Size distributions of females from all sampled areas demonstrate that relatively few of those completing the ovigerous period in the spring are present, after an increase in size, at spawning the following autumn. Most spawning females, their age then about 2½ years, had undergone sex change several months earlier.

The growth and reproduction of *Pandalopsis dispar* in southern British Columbia may be summarized. The eggs carried on the female hatch during March and April, and the pelagic life of five or six stages begins. Judging from the average size of post-larval individuals late in August, metamorphosis occurs

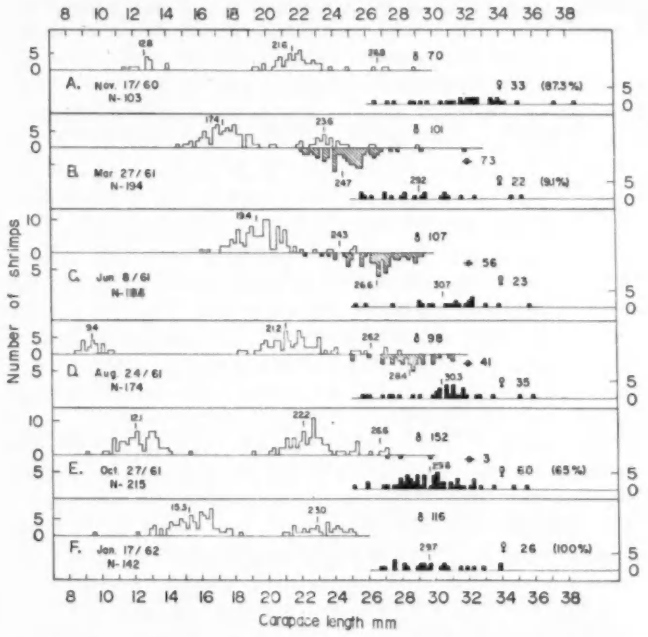


FIG. 7. ***Pandalopsis dispar.*** **Carapace length–frequency distributions from Howe Sound, November 17, 1960, to January 17, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.**

about late July or early August. These young males inhabit the same grounds as the older age groups. In Table III, mean carapace lengths, total lengths, and weights are tabulated for periods throughout the life of *P. dispar*. Total lengths and body weights were calculated from carapace lengths of Vancouver Island samples using the conversion factors (transitionals from male conversion factor) and equations presented earlier.

Males mature first in their second autumn, about 18 months following hatching. Sex change occurs the next spring when the shrimps are reaching 2 years of age, and most of the summer is required for completion of the four or five transition stages. By late October, in the third autumn, the shrimps, then females, are breeding and extruding eggs. The normal incubation period in southern British Columbia appears to be about 5½ months. Hatching begins in March and in most cases is completed before the end of April when the females are about 36 months old. Subsequently, the numbers of these females appear to diminish and it is assumed that relatively few survive into the fourth year.

This study reveals a different growth rate and age composition from that estimated by Berkeley (1930) but, in fact, her length frequency distributions

TABLE III. *Pandalopsis dispar*. Mean carapace lengths at age intervals from 5 to 38 months (based on samples from Vancouver Island, shown in Fig. 3), with mean total lengths and weights calculated from equations given in Tables I and II.

Month	Age (*in months*)	Sex	Mean carapace length (*mm*)	Mean total length (*mm*)	Mean total weight (g)
August	5	♂	9.3	58	0.52
October	7	♂	13.0	82	1.4
April	12	♂	17.3	109	3.3
October	19	♂	21.6	136	6.5
April	24	♂ (⊖)	25.0	145	10.1
August	29	Trans	27.1	156	12.9
October	31	♀	29.3	169	16.3
June	38	♀	30.4	176	18.2

resemble closely those of the present samples. The group of young males (mean 9.4 mm), in Fig. 6D corresponds to the group around 6 cm in total length measured by Berkeley from Howe Sound on August 29, 1929 (her fig. 26B). She correctly assumed that two other male age groups were present in this sample. One between 10.5 and 12.5 cm was about 16 months old; the other between 14 and 15 cm had individuals aged around 28 months. However, what was not evident was that these latter males, transitionals to 17 cm, and some females were the same age. Thus, because of the lack of sampling throughout the year, she had overestimated the ages of the several size and sex groups.

Pandalus jordani Rathbun

The range of *P. jordani* is from Unalaska to San Diego, 25–200 fathoms (45–358 m). Because of its lack of dorsal spines on the third and fourth abdominal segments it has been called "smooth pink" by some, although fishermen generally do not distinguish between it and *P. borealis*. Grounds where *P. jordani* is trawled are located in Barkley Sound and along the east coast of Vancouver Island (30 fathoms and deeper). Since 1952 the species has supported fisheries along the west coast of the United States from Morro Bay, California, to near Destruction Island, Washington. Fishable concentrations are located on the continental shelf from 3 to about 15 miles offshore, at depths between 50 and 100 fathoms, where the bottom material consists of green mud. At least one potential ground is known near Nootka Sound, off the west coast of Vancouver Island (Butler, 1959).

No description is available of the larval stages of *P. jordani*. Off the Washington coast, Tegelberg and Smith (1957) found that the ovigerous period lasted from late November to early April. Although details of growth and reproduction are unavailable, a summary of the life history, applicable to Oregon waters, was written by Ronholt and Magill (1961). They stated that *P. jordani* matures first as a male at approximately 18 months (shown in their graphs at

an average carapace length of 16.5 mm); then in the next year sex change occurs and the remainder of the life is spent in the female phase. Further, they mentioned the occasional occurrence of primary females.

Samples of *P. jordani* were gathered from two areas, the east coast of Vancouver Island, 25–110 fathoms (45–198 m) and Barkley Sound, 52–57 fathoms (94–103 m); all but 471 of the 6778 sexed and measured were from the former area.

Figure 8 shows the length composition of shrimps from the east coast of Vancouver Island, November 7, 1960, to January 19, 1962. The eggs carried by most females had hatched in late March and early April (Fig. 8C). and the larval life had begun. The group of smaller males (mean 8.2 mm) present in the late August sample had metamorphosed recently. During the next 2 months the growth rate of these males averaged about 1.8 mm per month (Fig. 8F). This group was of the same age, about 7 months, as the group (mean 11.6 mm)

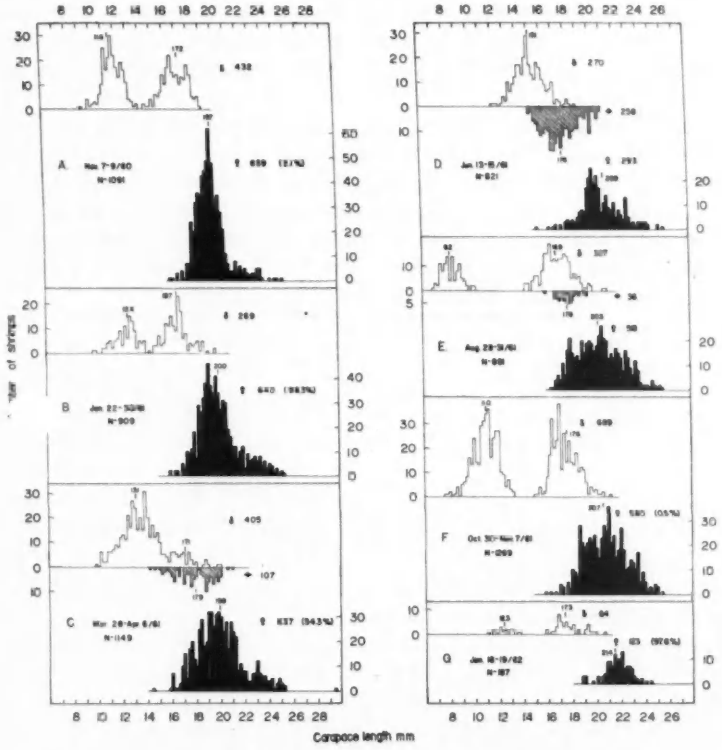
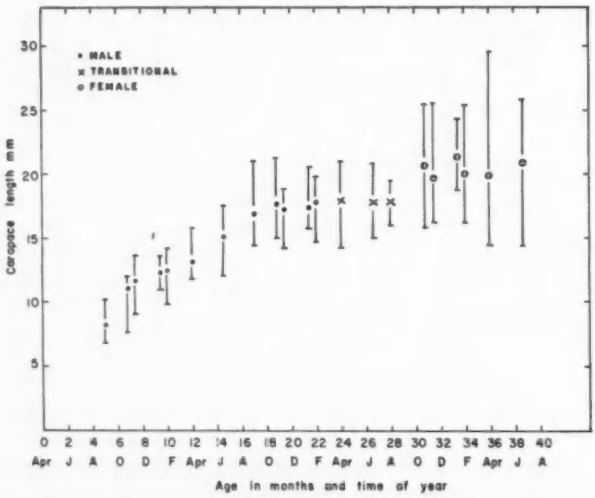


FIG. 8. *Pandalus jordani*. Carapace length–frequency distributions from east coast of Vancouver Island, November 7, 1960, to January 19, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

in Fig. 8A which was sampled a year earlier. The latter group was sampled again in January (Fig. 8B), and these males were present as yearlings in late March and early April (Fig. 8C). Growth continued through the summer (Fig. 8D, E), the increase averaging over 0.7 mm per month. By late October and early November (Fig. 8F) when this group (mean 17.6 mm) was about 1½ years maturity was reached. The corresponding group sampled early in November 1960 (Fig. 8A) was conspicuous again in the January sample (Fig. 8B), but was less so in late March and early April (Fig. 8C). Two-year-old individuals then appeared to be mainly in the transition phase. The change of sex continued until late summer (Fig. 8E). There was considerable overlapping of transitionals with yearling males (Fig. 8D), so it appeared that these also were undergoing sex change.

Age groups of *P. jordani* females were not clearly separated. The late August sample (Fig. 8E) showed an apparent recent influx of second- and third-year individuals which had completed sex transition. Two months later (Fig. 8F) there was still overlapping of age groups but females to 20.0 mm were assumed to be about 1½ years old; the ratio of females to males in this age group was 220 to 321. In all females at that time developing ova were conspicuous by the green colour of the ovary. In several samples (Fig. 8A, B, C) females between about 21 and 25.5 mm which were grouped to some extent were probably in their fourth year. The largest female, 29.3 mm, of the species ever measured, is graphed in Fig. 8C. Spawning began early in November (Fig. 8A, F) and hatching of eggs was well advanced by late March and early April (Fig. 8C).

The means and ranges of carapace lengths of *P. jordani* in samples graphed in Fig. 8 are summarized as a growth curve in Fig. 9. In Table IV, carapace and


• MALE
× TRANSITIONAL
○ FEMALE
Carapace length mm
30
25
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0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Apr J A O D F Apr J A O D F Apr J A O D F Apr J A
Age in months and time of year

FIG. 9. *Pandalus jordani*. Means and ranges of carapace lengths according to age, sampled from east coast of Vancouver Island.

TABLE IV. *Pandalus jordani*. Mean carapace lengths at age intervals from 5 to 38 months (based on samples from Vancouver Island, shown in Fig. 8), with mean total lengths and weights calculated from equations given in Tables I and II.

Month	Age (*in months*)	Sex	Mean carapace length (*mm*)	Mean total length (*mm*)	Mean tota weight (*g*)
August	5	♂	8.2	49	0.34
October	7	♂	11.0	66	0.85
February	10	♂	12.4	74	1.2
April	12	♂	13.1	78	1.9
August	17	♂ + (⊖)	16.9	101	3.3
October	19	♂ + (♀)	17.6	105	3.8
April	24	Trans (♀)	17.9	107	3.9
August	29	♀	20.2	118	5.8
June	38	♀	20.9	122	6.3

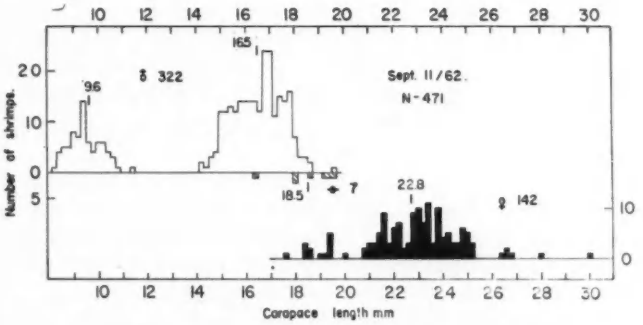


FIG. 10. *Pandalus jordani*. Carapace length–frequency distribution from Barkley Sound, September 11, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms.

total lengths and weights at intervals during its life are summarized. Because of overlapping of age groups the values plotted for females are less reliable than those during the male phase.

A single sample from Barkley Sound (Fig. 10) was collected on September 11, 1962. Small males, presumably the 0-group, had a mean length 9.6 mm. The rest (mean 16.5 mm) are believed to be second-year males then aged about 17 months. There were late-stage transitionals (mean 18.5 mm) which because of their overlapping in size range with the large males are presumed to be the same age. Females between 20.7 and 25.2 mm comprise a quite discrete group and then were likely about 29 months; but with only one sample it is uncertain whether or not other females belonged to the same age group. In the main, this distribution does not depart from the pattern seen in the late August sample (Fig. 8E) discussed earlier. Females on the average were larger, presumably

because of the scarcity of individuals which had undergone sex transition between 12 and 18 months.

In summary, the life cycle of *P. jordani* starts with the hatching of eggs from late March to early April in southern British Columbia and, following an unknown larval life, metamorphosis takes place probably early in August. Mean carapace lengths, total lengths, and weights are shown in Table IV for intervals from 5 to about 36 months after hatching. Normally individuals reach maturity first as males at 1½ years, but up to 40% of this age group may be mature females in the second autumn, having begun in the spring as immature yearlings to show change in external sex characters (Fig. 11). The proportion of transitionals among yearling shrimps appears to vary between regions, but

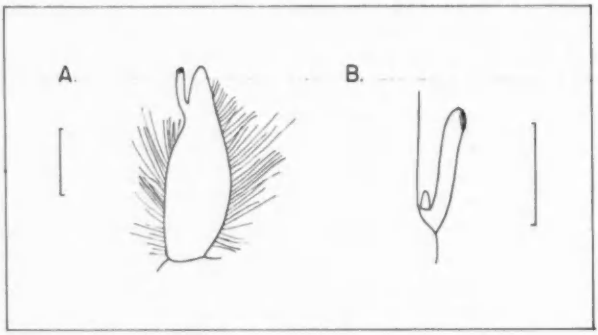


FIG. 11. *Pandalus jordani*. A—endopodite of first pleopod, and B—portion of endopodite of second pleopod of transitional individual 14.3 mm carapace length. Scale represents 1 mm.

so-called "primary" females were not recognized in present samples. Sex change of 2-year-old males is already in progress by early April and continues throughout the third summer. Spawning in southern British Columbia occurs during the last half of November and the ovigerous period lasts until April, a period of about 4½ months. Following hatching there is an increase in average length of females. Some appear to live into the fourth year and breed again but change in size distribution between spring and autumn indicates that most females die or at least disappear from the sampling area.

Comparison of present size distributions with available sampling data of *P. jordani* from Washington and Oregon indicates a similar age structure. In the distributions shown by Tegelberg and Smith (1957, their fig. 7) two groups of males appear in the March–April sample collected off Washington. At the time of sampling the group of smaller males presumably was 1 year old, and the larger ones 2 years. The female distributions in both samples are bimodal, but without year-round sampling it is uncertain whether two age groups were present. However, the wide overlapping in size between sexes suggests the presence of secondary females of the same age as the larger males, 1½ or 2 years depending on the time

of sampling, and having the same or a more rapid growth rate. The distributions figured by Ronholt and Magill (1961) show the male and female groups quite well separated. The average lengths of shrimps taken from the Coos Bay area (their fig. 5) do not differ appreciably from the means of corresponding groups in Vancouver Island samples at the same seasons.

Pandalus borealis Krøyer

The circumpolar distribution of *P. borealis* is outlined by Allen (1959): in the north Atlantic it occurs from Greenland to Cape Cod, and in European waters from Franz Josef Island to the North Sea. In the Pacific area the North American range is from the Bering Sea to the Columbia River, and along the Asian coast it is found as far south as the vicinity of Hokkaido. The bathymetric distribution in the Pacific is from 30 to 350 fathoms (53–630 m) (Rathbun, 1904). The local common name of this shrimp is "pink". Fishing areas in British Columbia waters are located in Burrard Inlet, Howe Sound, and Chatham Sound.

In Alaskan waters, Hynes (1929) found the average egg count based on 25 specimens was 2150. The complete larval life of six stages was described by Berkeley (1930). The collection of the first two stages from plankton samples in Burrard Inlet at about 50 fathoms (90 m) late in March and early April coincided with hatching of eggs by females. Later stages were taken rarely, and she concluded that at about the third stage the larvae migrate to shallower water, metamorphosing and spending the first summer there.

Early post-larval specimens, averaging 55 mm in total length, were found early in September in 25–35 fathoms (45–63 m). The subsequent development of *P. borealis* was determined by Berkeley as follows: during the next year individuals reach 95 mm and mature first as males; sex transition begins at about 2 years of age, and as females they spawn at the beginning of the third winter and may live for another year following the hatching of their eggs in the spring. Substantially the same cycle was reported for the species by Rasmussen (1953) and Allen (1959). The latter author found departures from the pattern of hermaphroditism in the presence of "primary" and "secondary" females, and demonstrated that the normal life span of *P. borealis* off the Northumberland coast of England is 3 years.

Samples of *P. borealis* (1894 specimens) taken by trawling in Indian Arm, a part of Burrard Inlet, 36–130 fathoms, between November 14, 1960, and January 16, 1962, are graphed in Fig. 12. The youngest specimens caught were three males (8.4–10.6 mm) in the October sample (Fig. 12E) which then were about 7 months old. More of the same age group (mean 11.1 mm) were present in the January sample (Fig. 12F). Yearling males (mean 12.8 mm) appeared in late March (Fig. 12B). Growth of males in the first part of their second year continued (Fig. 12C, D), about 0.5 mm per month; by late October at about 1½ years they had matured. Another group of second-year males was measured (mean 17.0 mm) in the November sample (Fig. 12A). By late March (Fig. 12B) few males remained and most individuals, then 2 years old, were in the

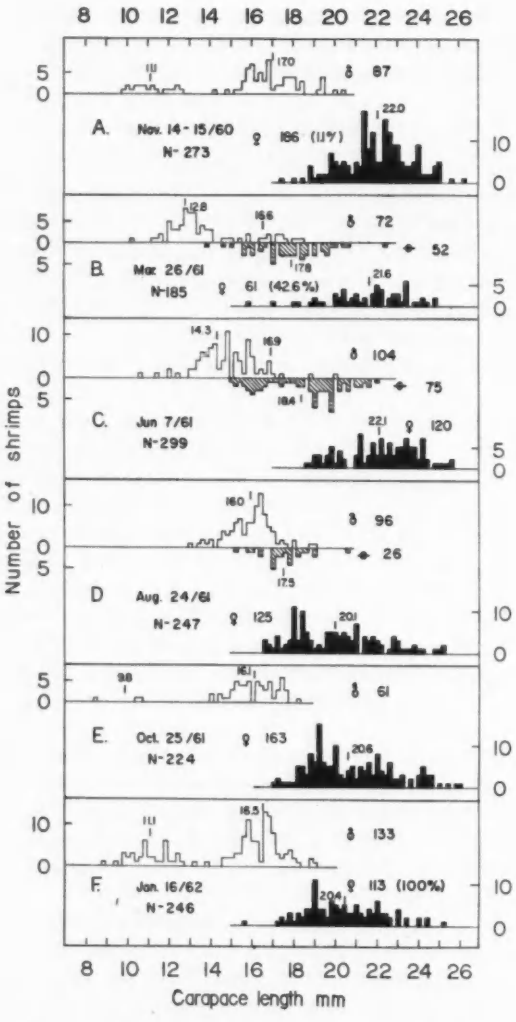
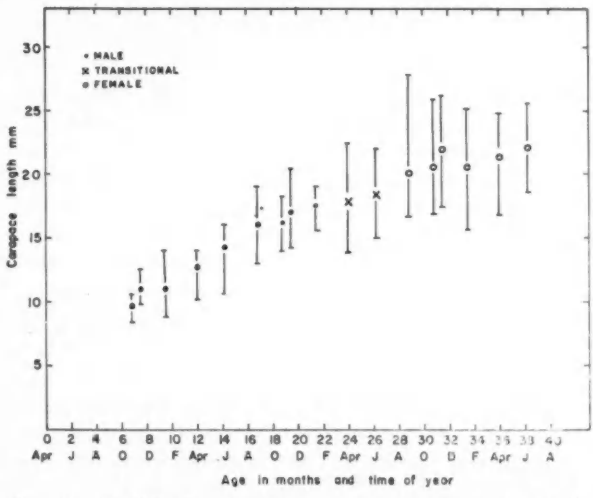


FIG. 12. *Pandalus borealis*. Carapace length–frequency distributions from Indian Arm, November 14, 1960, to January 16, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

transition phase (mean 17.8 mm). Sex change continued in June (Fig. 12C) and transitionals appeared to be comprised of two age groups. It is believed that those centred around 16.0 mm were yearlings because of their relative scarcity in March (Fig. 12B) in the age-two male and transition categories. Most females in the November sample (Fig. 12A) were considered to be normal hermaphroditic females, then around 2½ years old. Later in June (Fig. 12C) much the same grouping occurred and probably most females are beginning the fourth year. The August sample (Fig. 12D) showed the influx of individuals which recently had undergone sex change; an average decrease of 2.0 mm had occurred since June. The ratio of second-year females (estimated to 19.2 mm) to males of the same age was 51 to 96. There was a better separation of female groups in Fig. 12E: those centred around 19.0 mm were estimated to be about 1½ years, those around 22.0 mm a year older, and some 24.0 mm and larger were likely in their fourth year. In mid November (Fig. 12A) females were beginning to spawn and by late March (Fig. 12B) most had hatched their eggs.

Means and ranges of carapace lengths of *P. borealis* in Burrard Inlet samples appear as a growth curve in Fig. 13. In Table V a summary is given of lengths and weights attained at intervals throughout the life cycle.


• MALE
× TRANSITIONAL
o FEMALE
Carapace length mm
30
25
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0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Apr J A O D F Apr J A O D F Apr J A O D F Apr J A
Age in months and time of year

FIG. 13. *Pandalus borealis*. Means and ranges of carapace lengths according to age, sampled from Indian Arm.

The present sampling has confirmed Berkeley's growth and age estimates described earlier. Yearlings showing change of male external sex characters were found here but, like *P. jordani*, no primary females were evident. Secondary females were present and comprised about 47% of second-year individuals, averaged from the August and October samples. Although Berkeley found no mature individuals in the first-year group, some transition forms are graphed in her fig. 22.

TABLE V. *Pandalus borealis*. Mean carapace lengths at age intervals from 5 to 38 months (based on samples from Burrard Inlet, shown in Fig. 12), with mean total lengths and weights calculated from equations given in Tables I and II.

Month	Age (*in months*)	Sex	Mean carapace length (*mm*)	Mean total length (*mm*)	Mean total weight (g)
November	8	♂	11.0	65	1.2
April	12	♂	12.8	76	1.8
June	14	♂ + (⊖)	14.3	85	2.3
October	19	♂ + (♀)	16.1	95	3.2
April	24	Trans + (♀)	17.8	106	4.1
October	30	♀	20.6	119	6.1
June	38	♀	22.1	127	7.3

Rasmussen (1953) weighed 4143 specimens of *P. borealis* but did not include a length–weight equation in his paper. Present calculated weights, as given in Table V, are lower than those graphed in his fig. 6 but, because he used preserved shrimps which had been soaked in fresh water for 24 hr, the values probably are not comparable.

Pandalus platyceros Brandt

The range of this species is from Unalaska to San Diego, and off the coast of Japan from 2 to 266 fathoms. It is the largest of Pacific pandalids, and is known by British Columbia fishermen as the "prawn". Elsewhere the species has the common name "spot" shrimp because of the pair of white spots on the first and fifth abdominal segments. Because the prawn as an adult normally occurs on rocky bottom it is caught commercially by baited traps. Trapping grounds are located in the Strait of Georgia, along the west coast of Vancouver Island, and in most coastal inlets at depths from 40 to 90 fathoms.

Four larval stages and subsequent growth and migration of *P. platyceros* were described by Berkeley (1930). She found the first and second stages in deep water (unspecified depth, but presumably 50 fathoms and deeper), and specimens determined as the fourth and sixth stages were taken inshore at 2 or 3 fathoms. The presence of these late larvae and post-larval prawns in Departure Bay and other shallow inlets was evidence of metamorphosis there and subsequent habitation at least during the summer. A year after hatching, the prawns had migrated to deeper water (around 60 fathoms) and averaged about 10 cm in total length. Males were believed to be sexually mature in the second autumn, measuring about 15 cm. Because active males, 18–19 cm long, were found late in August, Berkeley assumed that another year was spent in the male phase. Then in the fourth spring, prawns underwent sex transition, having reached a total length of 20 cm; the life beyond that stage was undetermined. Females between 16.5 and 22.5 cm were measured but no mention was made of time of spawning or the ovigerous period. Near Petersburg, Alaska, Hynes (1929) found an average count of 3900 eggs carried by female *P. platyceros*.

For the present study a total of 8062 prawns was sampled from trawl and trap catches. In Fig. 14 the carapace lengths of 3231 prawns caught by trawl along the east coast of Vancouver Island are graphed. The earliest post-larval prawns (mean 14.4 mm) were present in the late August sample (Fig. 14E). Growth was very rapid during the next 2 months (Fig. 14F), an increase of over 1.9 mm per month. The corresponding age group had occurred (mean 19.0 mm) in the sample taken a year earlier (Fig. 14A). This latter group continued

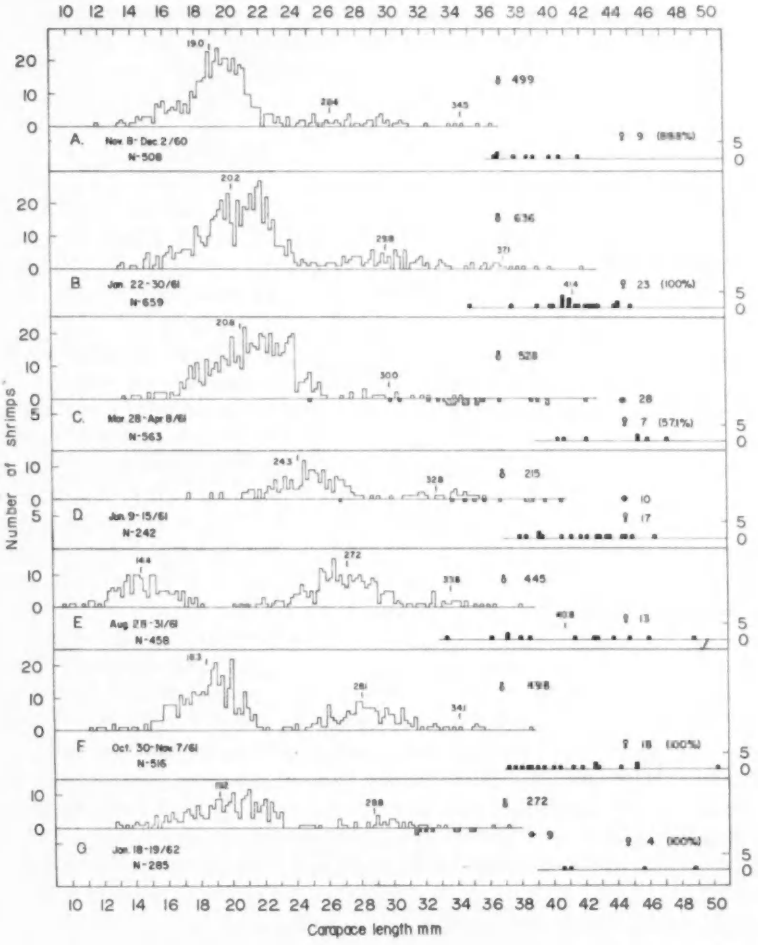


FIG. 14. *Pandalus platyceros*. Carapace length–frequency distributions, trawl-caught, east coast of Vancouver Island, November 8, 1960, to January 19, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

prominent in early 1961 (Fig. 14B) and by late March and early April these males had completed the first year of life. Through the following summer and early autumn a good growth rate continues (Fig. 14D, E, F), over 1 mm per month. In late October and early November these males, then mature, were aged about 1½ years. Later, by mid January (Fig. 14G), the mean of this group had increased slightly, and several of the larger individuals began to change sex. In the late January sample (Fig. 14B) taken a year earlier second-year males were well grouped (mean 29.8 mm), and 2 months later (Fig. 14C) this group had reached 2 years of age. A rapid growth rate occurred during summer months (Fig. 14D, E), and a few were in the transition phase (Fig. 14D). In late October and early November (Fig. 14F) the group, by then about 2½ years, was present in smaller numbers. This age group was present in Fig. 14A (mean 34.5 mm), and also in the late January sample (Fig. 14B). By late March and early April (Fig. 14C) those in the group, then aged 3 years, appear as transitionals.

Females were not numerous in most trawl-caught prawn samples, so age groups generally were not distinct. In one case (Fig. 14E), 6 females from 33.4 to 38.5 mm appeared to be in the first part of their third year and had changed sex recently (see Fig. 14D). Larger females in the same sample were likely a year older.

Another series of trawl collections from English Bay (25–90 fathoms) shown in Fig. 15, totalled 1103 prawns. In general the distributions resemble fairly closely those depicted in Fig. 14. Size ranges of age groups are much the same and differences between their means generally were 1 mm or less in favour of the English Bay samples; consequently, a detailed description is omitted here. It should be mentioned, however, that the lack or scarcity of first-year prawns in August, October, and November samples (Fig. 15E, F, A) in English Bay is due to the deeper trawling range there. By January (Fig. 15B, G) more had reached the sampling area from shallower water, the normal habitat of young prawns reported by Berkeley and borne out by the apparently representative sampling from Vancouver Island described earlier. Also evident from English Bay trawling, and consistent with earlier findings, was the presence of larger females; the mean carapace length of the 79 shown in Fig. 15 was 44.4 mm, and the mean of 91 measured (Fig. 14) from the other, generally shallower area was 41.6 mm.

In three other trawl samples, not graphed here, ovigerous females occurred as follows: September 11, 1962, Barkley Sound, 52–57 fathoms, (N = 26) 4 females, 41.0–48.6 mm, ovigerous; October 10, 1962, English Bay, 50 fathoms, (N = 15) 2 females, 43.7 and 49.3 mm, ovigerous; October 11–16, 1962, east coast of Vancouver Island, 13–68 fathoms, (N = 434) 7 females, 36.6–48.5 mm, ovigerous.

Trap fishing for prawns carried out on grounds off Snake Island and Five Finger Island (in the Strait of Georgia near Nanaimo), from 30 to 84 fathoms, resulted in the collection of 3728 specimens which were graphed in Fig. 16. Older males and females were more numerous than in trawl samples (Fig. 14). In the first trap sample (Fig. 16A) second-year (mean 26.6 mm) and third-year

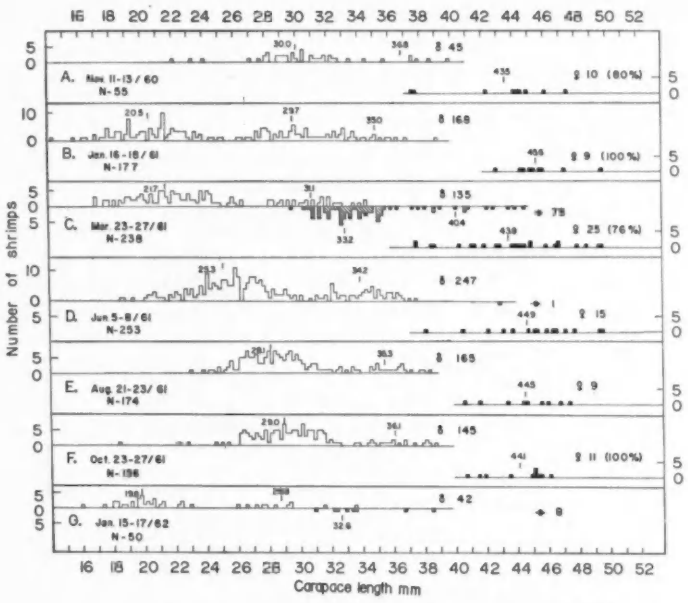


FIG. 15. *Pandalus platyceros*. Carapace length–frequency distributions, trawl-caught, English Bay, November 11, 1960, to January 17, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

males (mean 32.9 mm) were in conspicuous groups with some overlapping. The former group could be followed through the winter (Fig. 16B), and by early April (Fig. 16C) were 2 years old (mean 28.1 mm); 2-year-old transitionals (mean 30.7 mm) were also present. Sex change in this group continued during the summer (Fig. 16D, E) and apparently gave rise to females such as those depicted in Fig. 16E and F, between 31.8 and 34.3 mm. The third-year individuals in January (Fig. 16B) are males but in April (Fig. 16C) practically all were in the transition phase (mean 35.6 mm). Taking into account the size range of most females and their presence in numbers in the late summer and autumn (Fig. 16A, E), it was concluded that these were in the fourth year and had undergone sex change in preceding months. In November (Fig. 16A, F) most, if not all, females had spawned and by early April (Fig. 16C) the hatching of larvae was well advanced.

The means and ranges of carapace lengths as found in trawl and trap samples from the east coast of Vancouver Island are plotted as a growth curve for *P. platyceros* in Fig. 17; in Table VI, carapace lengths, total lengths, and weights at intervals during its life are summarized. In general the features of this species' biology as outlined here agree with Berkeley's findings. Prawns during the

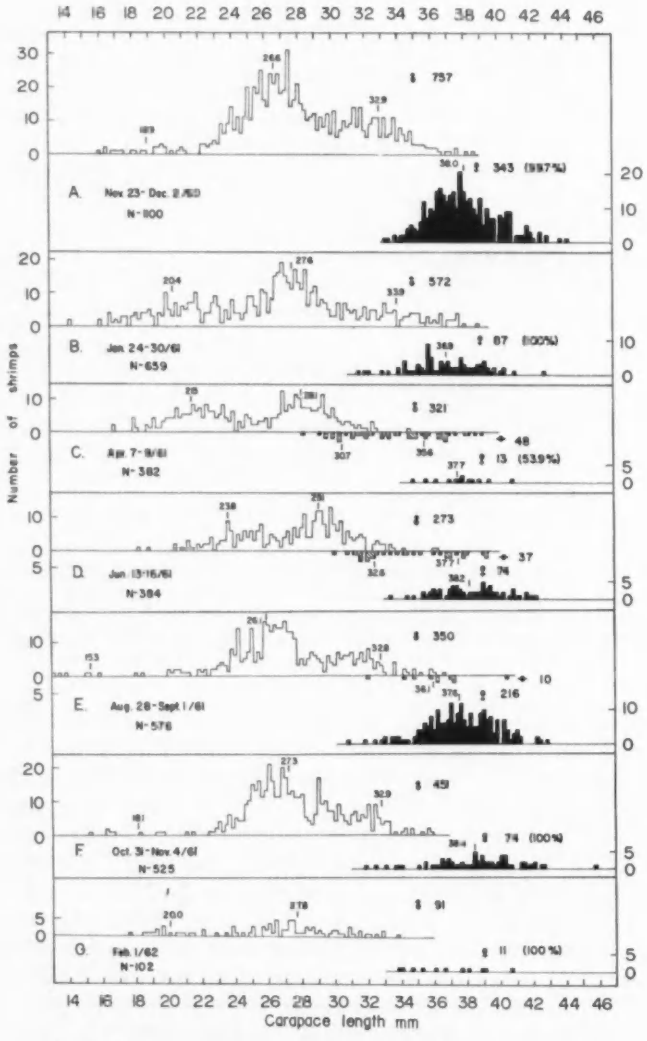
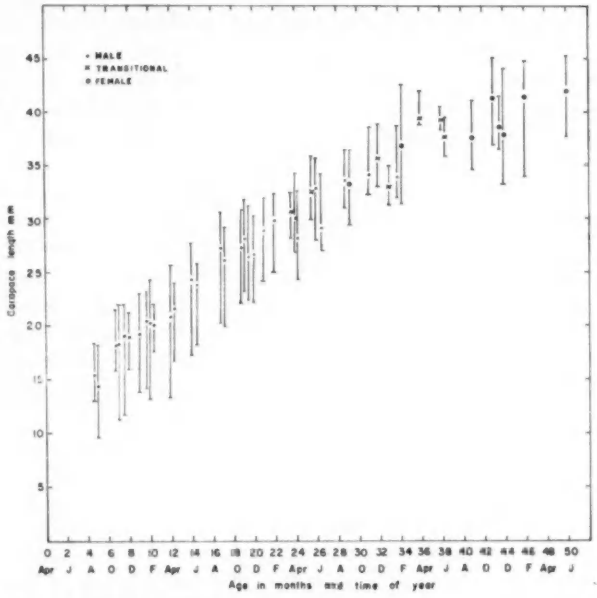


FIG. 16. *Pandalus platyceros*. Carapace length–frequency distributions, trap-caught, east coast of Vancouver Island, November 23, 1960, to February 1, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.


• MALE
× TRANSITIONAL
○ FEMALE
Carapace length mm
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0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
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Age in months and time of year

FIG. 17. *Pandalus platyceros.* Means and ranges of carapace lengths according to age; combined samples, trawl and trap.

TABLE VI. *Pandalus platyceros.* Mean carapace lengths at age intervals from 5 to 50 months (based on samples from Vancouver Island, shown in Fig. 14 and 16), with mean total lengths and weights calculated from equations given in Tables I and II.

Month	Age (*in months*)	Sex	Mean carapace length (*mm*)	Mean total length (*mm*)	Mean total weight (*g*)
August	5	♂	14.4	73	2.1
November	7	♂	18.3	92	4.2
April	12	♂	21.2	107	6.4
August	17	♂	26.7	135	12.7
November	19	♂	27.7	140	14.2
April	24	♂ + (⊖)	28.5	144	15.4
November	31	♂	32.9	166	23.4
November	31	♀	33.2	158	24.1
April	36	Trans	33.7	170	25.1
November	42	♀	37.9	180	35.5
June	50	♀	42.1	200	48.3

first year after hatching normally frequent shallower regions, 30 fathoms or less, but small numbers in trap sets indicate metamorphosis of some in relatively deep water. The male phase continues into the third year but not for all individuals as some are in the transition phase during their third summer. This point did not become evident during the earlier work. At the end of 3 years all males have either completed or are undergoing sex change. Thus, third- and fourth-year females are mature in the rocky trapping regions. Following hatching of eggs in the spring, practically all females evidently leave these grounds and the conclusion is that most die. Rarely do these individuals reappear in traps later in the summer, when after moulting, they probably range between 43 and 50 mm in carapace length. The mean length of all trap-caught females was 37.6 mm. As was mentioned above, more large individuals were found on deeper trawling grounds. These large females are presumably fast-growing individuals in the fourth year or others in their fifth year. Normally spawning is over at the end of October and the ovigerous period lasts between 5 and 5½ months.

Pandalus hypsinotus Brandt

This shrimp is known from the Bering Sea to the Strait of Juan de Fuca, and in Asian waters along the Kurile Islands and Hokkaido, Japan. The bathymetric range given by Rathbun (1904) was 3–20 fathoms but along the British Columbia coast it is fished between 20 and 40 fathoms and specimens have been collected from 70 fathoms. Commercially, *P. hypsinotus* is of minor importance, entering trawl catches along with *P. jordani*, *P. borealis*, and *P. danae* in such areas as Stuart Channel, Masset Inlet, and Burrard Inlet. The arched shape of the dorsal surface of the carapace gives this species its common name of "humpback".

The main features of the biology of *P. hypsinotus* were reported by Berkeley (1930). The first-stage larva was hatched in the laboratory but no later stage was determined definitely. Second- and third-stage larvae resembling *P. danae*, but assumed to be *P. hypsinotus*, were collected in the same habitat as the adults. Limited sampling suggested that metamorphosis occurred in shallower water, 20–30 fathoms, and that young shrimps lived there at least part of the first year. As yearlings (March–May), males were found to average 9.0 cm in total length and by fall, at 11 cm, were active sexually. The presence of transition individuals, between 9 and 13.5 cm, in spring samples indicated that sex change began around 2 years of age. It was evident that most females matured at 2½ years, averaging about 14 cm early in September, and lived at least another 6 months until eggs were hatched. Some females in Berkeley's September sample (her fig. 24C), from 11 to 12.5 cm, overlap the male group and presumably these smaller individuals were mature at 1½ years. Ovigerous females hatching eggs were found during April, but no statement regarding the period of spawning in the fall was given.

Igarashi (1951) in a study at Hokkaido concluded that *P. hypsinotus* matured as a male at 2 years, changed sex at 3 years, and lived another year during which

it produced a brood of eggs. The ovigerous period extended from September to March, and average egg counts for females from 10.2 to 15.0 mm in carapace length were 264 to 3000. For this species, Hynes (1929) found in Alaskan waters an average number, based on five females, of 4000 eggs.

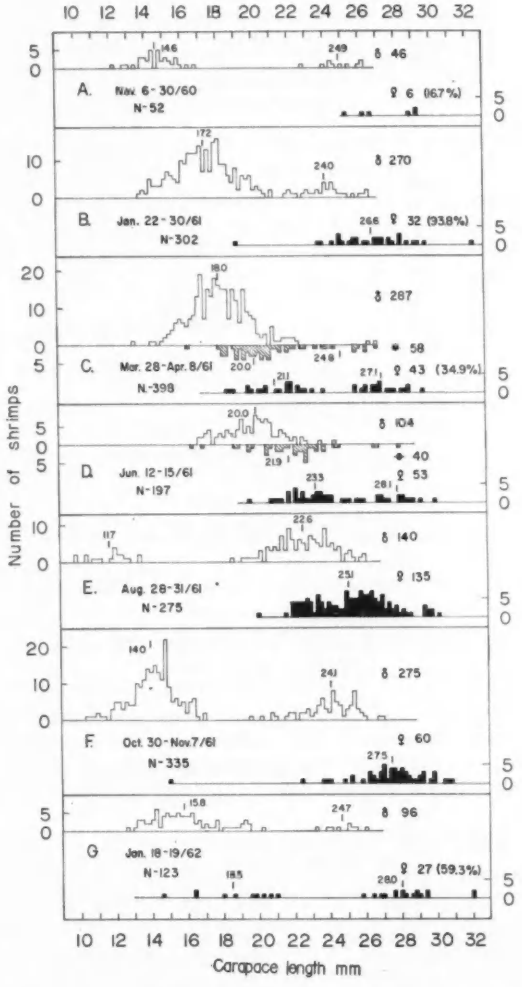

FIG. 18. *Pandalus hypsinotus*. Carapace length–frequency distributions, east coast of Vancouver Island, November 6, 1960, to January 19, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

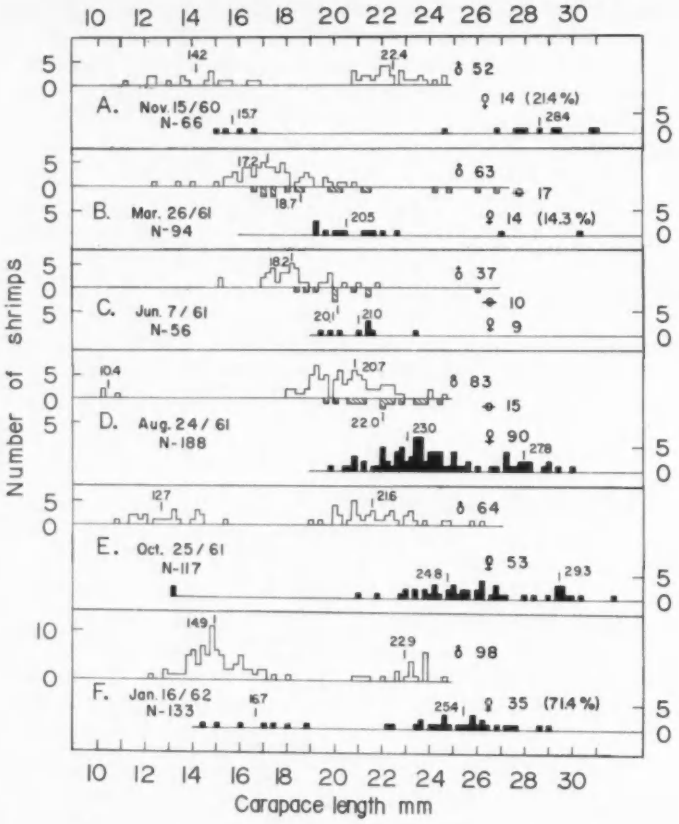

FIG. 19. *Pandalus hypsinotus*. Carapace length–frequency distributions, Burrard Inlet, November 15, 1960, to January 16, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

Samples totalling 2336 specimens were collected by trawling. In Fig. 18 are graphed the 1682 shrimps caught along the east coast of Vancouver Island. The youngest *P. hypsinotus* collected, about 5 months old (mean 11.7 mm), appeared in late August (Fig. 18E). Growth rate was rapid for the next 2 months (Fig. 18F), about 1.1 mm per month. The corresponding age group was caught approximately a year earlier (Fig. 18A), and remained conspicuous through the winter (Fig. 18B) until late March and early April (Fig. 18C) when it was 1 year old. The average increase per month was fairly constant, about 0.9 mm, during the summer and autumn (Fig. 18D, E, F). These males were then about 1½ years old and were sexually mature. The group persisted (mean 24.7 mm) in the January sample (Fig. 18G); its counterpart a year before appeared

in Fig. 18B. By late March and early April (Fig. 18C) most individuals of the group, then 2 years old, appeared to be in the transition phase or already had changed sex. The same was evident with yearlings then which consisted of males, transitionals (mean 20.0 mm), and non-ovigerous females (mean 23.3 mm), and this picture persisted with size increases until mid June, at least. In late August (Fig. 18E), the considerable overlap of the female size distribution with the male group indicated the presence of both second- and third-year females. Primary females were identified in two samples (Fig. 18F, G) and evidently were immature because none was ovigerous. The growth rate of these females appeared faster than males of the same age.

Another series of samples of 654 *P. hypsinotus* collected in Burrard Inlet, 11–52 fathoms, is graphed in Fig. 19. Since essentially the same pattern is seen as in the Vancouver Island series, this one will not be outlined in detail. Primary females appeared also in Burrard Inlet distributions (Fig. 19) in proportion to males as follows:

Date	*Males*	*Females*
November 15, 1960	19	4
October 25, 1961	23	2
January 16, 1962	78	7

These females averaged 1.2 mm larger than males of the same age, and were sexually inactive.

On August 24, 1961 (Fig. 19D), in the second-year group the ratio of mature females (19.6–25.7 mm) to males (17.9–24.6 mm) was 71 to 80, and potentially higher if transitionals are included; on October 25 (Fig. 19E) the ratio of females (20.7–27.2 mm) to males (18.7–25.9 mm) was 38 to 41. The transition period appeared to last longer in the Burrard Inlet area (compare Fig. 19D and 18E).

In most cases means of carapace lengths of age groups in the Vancouver Island samples were higher: first-year males, 0.2–1.3 mm; second-year males, 1.8–2.5 mm; transitionals, 1.7–1.8 mm; and females, 0.6–2.3 mm. However, the value of these comparisons is somewhat lessened due to the smallness of most Burrard Inlet samples.

Figure 20 shows means and ranges of carapace lengths of *P. hypsinotus* samples from the east coast of Vancouver Island and Burrard Inlet. Table VII summarizes the mean lengths, carapace and total, and total weights of *P. hypsinotus* at intervals during its life in southern British Columbia. The present results agree well with the picture of the life history, though incomplete, described by Berkeley. Not evident previously is the presence of primary females which comprise from 8 to 21% of individuals of that age group and are larger than males, maturing like them in the second autumn. More numerous are individuals which apparently are not active as males and undergo, at 1 year of age, a change in external characters. Those remaining as males are sexually active at about 1½ years, and change sex the following spring.

The paucity of large females suggests that survival is low following egg hatching at 3 years. The spawning period is not clearly defined by present observations but is likely completed by early December; the ovigerous period then lasts no longer than 4½ months.

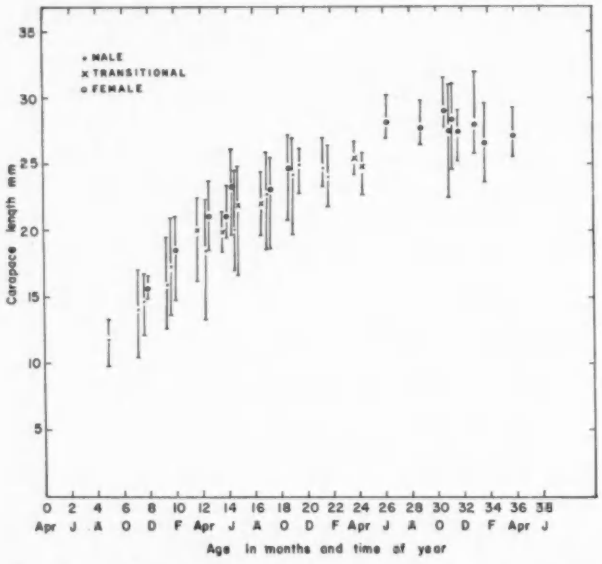
• MALE
x TRANSITIONAL
o FEMALE
Carapace length mm
35
30
25
20
15
10
5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Apr J A O D F Apr J A O D F Apr J A O D F Apr J
Age in months and time of year

FIG. 20. *Pandalus hypsinotus*. Means and ranges of carapace lengths according to age, combined samples.

TABLE VII. *Pandalus hypsinotus*. Mean carapace lengths at age intervals from 5 to 36 months (based on samples from Burrard Inlet and Vancouver Island shown in Fig. 18 and 19), with mean total lengths and weights calculated from equations given in Tables I and II.

Month	Age (*in months*)	Sex	Mean carapace length (*mm*)	Mean total length (*mm*)	Mean total weight (*g*)
August	5	♂	11.7	60	1.4
November	7	♂	14.0	72	2.3
April	12	♂ + (⊖)	17.4	89	4.1
April	12	♀	21.1	106	6.3
June	14	♂ + (⊖)	20.0	103	6.1
June	14	♀	23.3	117	9.3
November	21	♂ + (♀)	24.1	124	10.3
April	24	♂ + (⊖)	25.0	129	11.4
April	24	♀	25.0	125	11.4
November	31	♀	26.0	130	12.7
April	36	♀	27.5	138	15.1

Pandalus danae Stimpson

This shrimp derives its local common name of "coonstripe" from the irregular brown or red striping on the abdomen. In other Pacific coast regions it is often known as the "dock" shrimp. The known distribution is from Sitka, Alaska, to San Luis Obispo Bay, California, 10–101 fathoms (Goodwin, 1952). *Pandalus danae* is found on sand or gravel, usually where a rapid tidal current exists. The only trawling ground off British Columbia where the species is at all abundant is located in Burrard Inlet near Vancouver.

Sex reversal in pandalid shrimps was first observed in *P. danae* by Berkeley (1929a). In a subsequent investigation (1930) she worked out the larval as well as the adult life of this species fairly completely. In addition to her studies on the five larval stages, she measured and weighed over 2500 shrimps from six bi-monthly hauls.

All larval stages occurred near the place of hatching, at 10–30 fathoms, nearing the bottom as development progressed. By late June, metamorphosis was complete, and growth was rapid until October when at 6 or 7 cm in total length, the shrimps became active males. During the next year individuals remained as males and were found to reach about 8.5 cm by the second autumn. Sex change reportedly occurred in the third spring, and in October, females spawned at about 10 cm. The subsequent life of 3-year-old females after egg hatching in March and April was uncertain.

The series of 1126 *P. danae* sampled from Burrard Inlet, 10–36 fathoms, is graphed in Fig. 21. Probably the most striking feature of these six samples, collected throughout the year, is that all contain ovigerous females. Although the percentages were fairly low during the summer season (Fig. 21C, D), these never approached in winter months (Fig. 21A, F) the 100% value normal for other species. That breeding evidently is more or less continuous is reflected in the almost complete lack of grouping in size distributions of both sexes.

In Fig. 21A carapace lengths of males ranged from 9.8 to 21.0 mm, but individuals to about 14.0 mm (mean 12.9 mm) appear to belong to a single age group. A clearer separation existed in the late October sample (Fig. 21E) of males between 9.9 and 14.6 mm, mean 12.8 mm. These groups obviously correspond to males averaging 6.5 cm in total length in Berkeley's October collection which she concluded were 0-group individuals. Also in Fig. 21E, larger males between 15.3 and 21.0 mm, mean 17.3 mm, correspond to the second-year males, at about 8.5 cm, present in the October sample in 1928.

The mean lengths of males at 1 and 2 years are uncertain. In Fig. 21B, transitionals form a group between 12.4 and 16.9 mm, mean 15.6 mm, and presumably are yearlings; males may be expected to average the same or less. Again in the same collection 2-year males may be represented by those from 17.2 to 21.7 mm, mean 18.7 mm; 9 transitionals from 17.5 to 19.2 mm had a mean of 18.5 mm. There was a fairly distinct group of males in Fig. 21C, between 17.7 and 20.8 mm, mean 19.3 mm, which likely was about 26 months old; the corresponding group, between 17.5 and 21.9 mm, undergoing sex change averaged 19.4 mm.

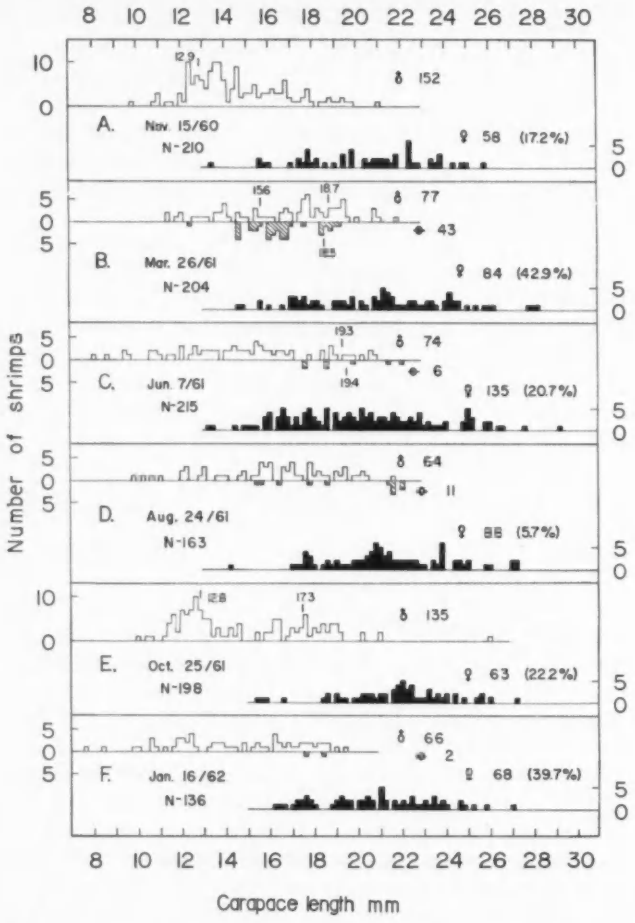

FIG. 21. *Pandalus danae*. Carapace length–frequency distributions, Burrard Inlet, November 15, 1960, to January 16, 1962. Length data are given in 0.2 mm divisions. Average lengths are shown for individual histograms. Percentage of females that were ovigerous is indicated in parentheses.

In all the samples considerable overlap existed between male and female distributions. It is not clear whether primary females are present in *P. danae* populations. Several of the present samples (Fig. 21A, C, D) had isolated specimens between 13 and 14 mm, and also several individuals at 6 and 7 cm in total length appearing in Berkeley's collections may have been primary females. However, there is no doubt that many individuals after a year have a change in

external characters and mature as females at 1½ years, probably in most cases in the second autumn. Sex change of second-year males begins as early as January (Fig. 21F) and continues throughout the third spring and summer. In the fall the third-year females, then active, may average 21.7 mm (Fig. 21E).

The means and ranges of carapace lengths given above for *P. danae* are summarized in Fig. 22; total lengths and body weights, calculated from these means, at intervals during the life, are presented in Table VIII. The calculated weights here agree well with those obtained directly by Berkeley (her table I, 1930).

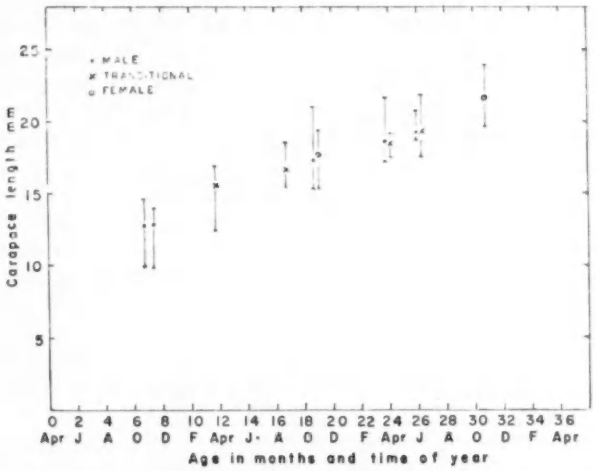
MALE
TRANSITIONAL
FEMALE
Carapace length mm
25
20
15
10
5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Apr J A O D F Apr J A O D F Apr J A O D F Apr
Age in months and time of year

FIG. 22. *Pandalus danae.* Means and ranges of carapace lengths according to age, sampled from Burrard Inlet.

TABLE VIII. *Pandalus danae.* Mean carapace lengths at age intervals from 7 to 31 months (based on samples from Burrard Inlet, shown in Fig. 22), with mean total lengths and weights calculated from equations given in Tables I and II.

Month	Age (*in months*)	Sex	Mean carapace length (*mm*)	Mean total length (*mm*)	Mean total weight (*g*)
October	7	♂	12.8	65	1.8
April	12	♂ + (⊖)	15.6	79	3.2
October	19	♂	17.3	88	4.4
October	19	♀	18.0	87	4.9
April	24	♂ + (⊖)	18.7	95	5.5
June	26	♂ + (⊖)	19.3	98	6.0
October	31	♀	21.7	105	8.5

Pandalus montagui tridens Rathbun

The range of this subspecies is from the Bering Sea to off San Nicolas Island, California, 3–1084 fathoms (Schmitt, 1921). The normal habitat is uncertain, during the present study specimens were caught in prawn traps as well as by trawl hauls on mud bottom. *Pandalus montagui tridens* resembles *P. jordani* in body form and size but, whereas *P. jordani* when alive is a uniform light pink, the former species is an orangish-red with yellow blotches over the body and appendages.

Nothing has been written on the biology of the Pacific subspecies but the Atlantic form, *P. montagui* Leach, was investigated fully by Mistakidis (1957). He confirmed the earlier reports of sex reversal and the existence of primary females, and determined growth rate and ecology of this shrimp in the vicinity of the Thames River estuary. Squires (1961) sexed and measured specimens obtained during exploratory shrimp fishing in Newfoundland waters.

Information on *P. montagui tridens*, as well as the two other uncommon species, *P. stenolepis* and *P. goniurus*, is rather fragmentary. However, the data collected shed light on the question of hermaphroditism in these pandalid shrimps. In Fig. 23 are diagrams of the endopodites of the first pleopod, and parts of the same ramus on the second pleopod of specimens of *P. montagui tridens* taken from Departure Bay in August 1961. The endopodite (Fig. 23A) of an active male, 11.6 mm, had at its apex a copulatory organ fringed with the small hooked cincinnuli. The portion of the endopodite of the second pleopod (Fig. 23A′) from the same male had the appendix interna with cincinnuli on its outer margin, and the appendix masculina armed with 7 strong spines (6 shown in Fig. 23A′, and several appear broken).

The endopodite of a specimen 12.1 mm (Fig. 23B) had a more slender apex and the copulatory organ was undergoing atrophy. The appendix masculina of the second pleopod (Fig. 23B′) from the same transitional individual was much reduced and without spines. The female phase was exemplified by the slender tapering endopodite (Fig. 23C) from a specimen 15.4 mm. The ovigerous setae were well developed. The appendix masculina had disappeared from the endopodite of the second pleopod (Fig. 23C′) of the same female, and the appendix interna was stouter and bore more cincinnuli than during male or transition phases.

In Fig. 24 are graphed four small samples, totalling 256 specimens, of *P. montagui tridens* collected near Nanaimo during 1961. The first sample (Fig. 24A) on January 26, taken from two trawl hauls off Snake Island at 49–50 and 110 fathoms, consisted of 12 ovigerous females, 14.8–18.9 mm. Between April 5 and 7, 22 specimens (Fig. 24B) were obtained from prawn trap sets, 40–92 fathoms, near Snake Island and Five Finger Island, and two trawl tows near Snake Island, at 106–110 and 50–55 fathoms, respectively. Three males, 12.0–12.8 mm, were present, and 19 females ranged from 15.0 to 19.4 mm; 5 were ovigerous. The next sample (Fig. 24C), obtained on August 31 from two tows near Snake Island at 48–50 fathoms, and in Departure Bay, 20–25 fathoms, had a male distribution from 11.4 to 13.5 mm, mean 12.1 mm. It seems unlikely that these males belonged to the 0-group which hatched earlier in the spring so

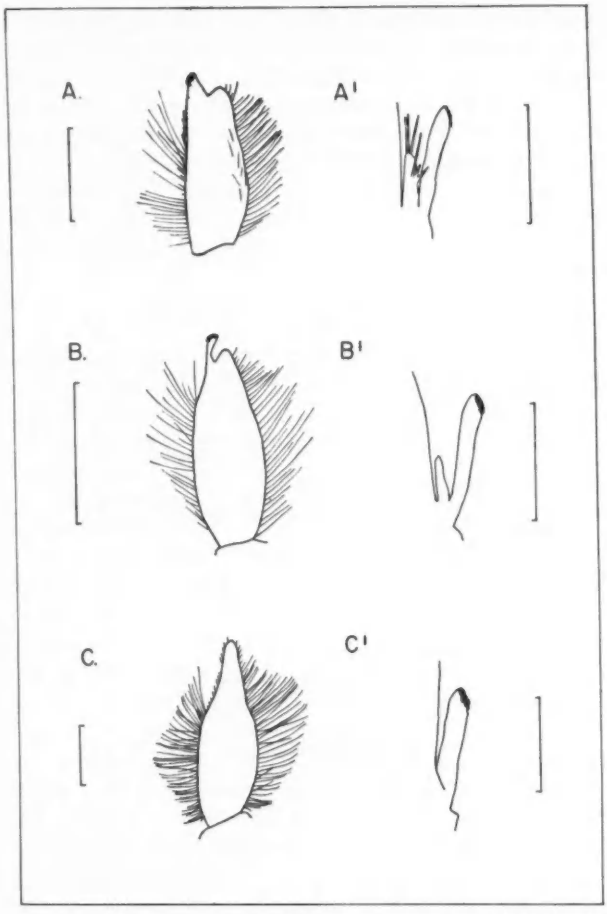

FIG. 23. *Pandalus montagui tridens*. A—endopodite of first pleopod and A'—portion of endopodite of second pleopod of male with carapace length of 11.6 mm. B, B'—same structures of transitional individual with carapace length of 12.1 mm. C, C'—same structures of female with carapace length of 15.4 mm. Scale represents 1 mm.

the assumption is that they were about 1½ years old, hatching in 1960. The two transitionals, at 12.8 and 13.5 mm, appeared to be the same age as the males.

The collection from November 2 to 7 (Fig. 24D) was obtained off Snake Island in trap sets at 50 fathoms and from a trawl tow at 48–50 fathoms. Second-year males extended from 11.4 to 15.0 mm, mean 12.6 mm, and average increase since August was 0.5 mm. This apparent low increment is taken as evidence that these males did not belong to the 0-group. Females ranged from 13.9 to 20.2 mm, and no ovigerous individual was found. Seven other specimens

have been collected elsewhere. A male, 13.4 mm in carapace length, was trawled in Howe Sound at 140 fathoms on October 27, 1961. On June 5, 1961, two females, 14.8 and 15.0 mm, were taken in a trawl haul in English Bay, at 20–21 fathoms. Several tows in Chatham Sound at 30–58 fathoms on July 8, 1961, yielded 4 males, 9.0–9.5 mm, mean 9.1 mm; it is uncertain whether these latter males were recently metamorphosed or in their second year.

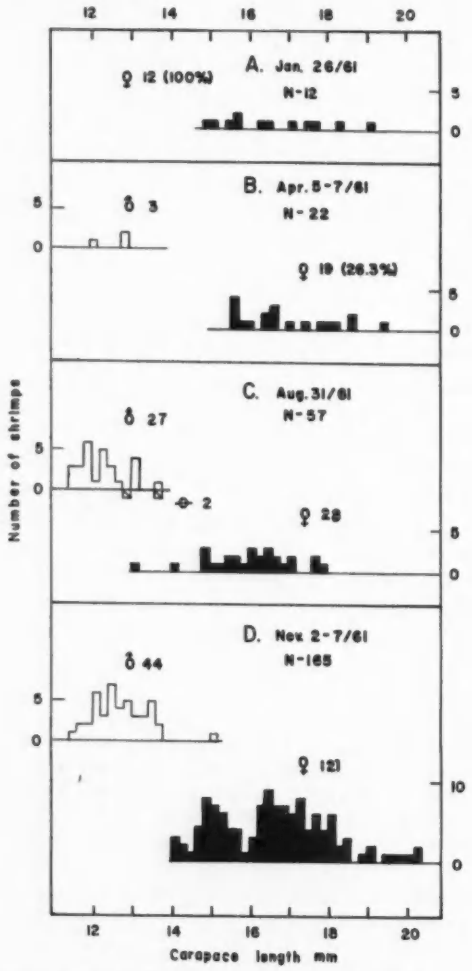

FIG. 24. *Pandalus montagui tridens*. Carapace length–frequency distributions, east coast of Vancouver Island, January 26 to November 7, 1961. Length data are given in 0.2 mm divisions. Percentage of females that were ovigerous is indicated in parentheses.

Though present measurements are limited, it is clear that growth and reproduction of *P. montagui tridens* are dissimilar to the European *P. montagui* as reported by Mistakidis (1957). The Pacific shrimp is hermaphroditic, but no primary females were found. There is an indication that some individuals of *P. montagui tridens* change sex before their second autumn (Fig. 18C), but normally males are active at 1½ years. Males of *P. montagui* undergo sex change at one year or so, and most apparently end the second year as active females. Thus, females of the latter species are on the average smaller than the Pacific subspecies which likely has a longer life span, to 4 years.

Pandalus stenolepis Rathbun

This species appears to be the rarest of shrimps of the genus *Pandalus* occurring on the British Columbia coast. The entire recorded distribution is from the Aleutian Islands to Oregon, 27–125 fathoms. Specimens have been caught in prawn traps and in trawl hauls on mud bottoms but most often when sponges (*Rhabdocalyptus dawsoni* and *Aphrocallistes vastus*) were present. *P. stenolepis* is a stout species, having the sharply ascending rostrum with the tip usually bifid, and rarely trifid. Its brown and red colouring is similar to *P. danae* but it appears as blotches rather than striping. Small blue spots, 1–3, are present on the posterior dorsal part of the carapace and on the dorsal surfaces of the first to fifth abdominal segments. Needler (1938) described six larval stages and the first post-larval stage.

Diagrams of the external sex characters of *P. stenolepis* at different sexual phases appear in Fig. 25. The endopodite of an active male (Fig. 25A), 10.4 mm, had at the tip the prominent copulatory organ which bore cincinnuli. This specimen was dredged in Narvaez Bay near Saturna Island, at 40 fathoms on August 24, 1961. The endopodite of the second pleopod from this male (Fig. 25A′) had, in addition to the outer appendix interna, an elongated appendix masculina armed with three rigid spines at the tip and about a dozen smaller spines along the inner margin.

The endopodite of a transitional shrimp, 11.4 mm carapace length (Fig. 25B), collected from Chatham Sound on July 8, 1961, was elongate with the shrivelled copulatory organ carrying only several cincinnuli. Little of the appendix masculina of the same transitional (Fig. 25B′) remained; all spines had disappeared and one seta was at the tip of the diminished appendage. The first endopodite (Fig. 25C) of a mature female, 15.0 mm, from Departure Bay on October 12, 1962, was slender, nib-like, and bore ovigerous setae. On the second pleopod the appendix masculina was absent (Fig. 25C′); the appendix interna was enlarged and cincinnuli around the tip were numerous.

The distributions shown in Fig. 26 are of *P. stenolepis* collected in Chatham Sound, 30–58 fathoms. The first sample (Fig. 26A) of 32 specimens shows 5 males, from 8.0 to 11.0 mm. One individual in the transition phase, at 11.0 mm, was present, and females ranged from 9.9 to 16.9 mm.

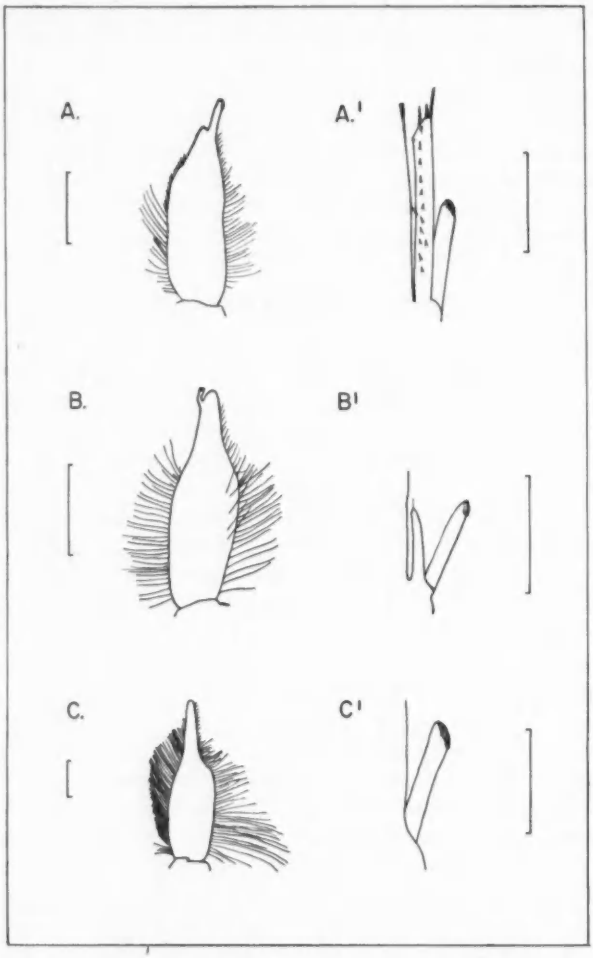

FIG. 25. *Pandalus stenolepis*. A—endopodite of first pleopod and A'—portion of endopodite of second pleopod of male with carapace length of 10.4 mm. B, B'—same structures of transitional individual with carapace length of 11.4 mm. C, C'—same structures of female with carapace length of 15.0 mm. Scale represents 1 mm.

The other collection (Fig. 26B) taken in the same month 2 years later showed no essential differences; 10 males ranged from 8.5 to 10.5 mm, and those of 7 transitionals from 9.4 to 11.8 mm. The size range of females was less, from 12.3 to 15.1 mm.

Two other small collections should be mentioned: 5 females taken in a trawl haul near Snake Island at 48–50 fathoms on November 7, 1961, ranged

from 10.9 to 15.7 mm and none was ovigerous; the other group of 6 females, measuring from 11.2 to 13.2 mm, was collected by trawl in Departure Bay at 20–25 fathoms, on April 5, 1961 (one of the latter females, at 12.2 mm, carried "eyed" eggs). Thus, it appears that the ovigerous period of this species does not begin in southern British Columbia before late November and ends early in April.

Little can be summarized about the biology of *P. stenolepis*. Growth rate and age composition are unknown. The scanty data here show that the shrimp is hermaphroditic and like most of British Columbia pandalids it undergoes sex transition during the summer season, with spawning and egg-bearing confined to winter months.

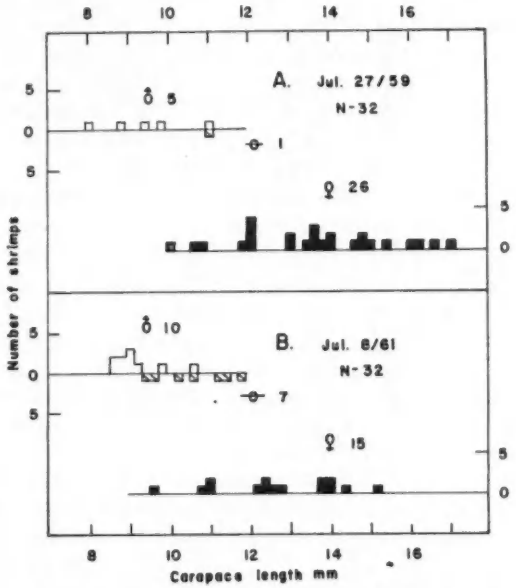

FIG. 26. *Pandalus stenolepis*. Carapace length–frequency distributions, Chatham Sound, July 27, 1959, and July 8, 1961. Length data are given in 0.2 mm divisions.

Pandalus goniurus Stimpson

The range of this shrimp along the North American coast is from Arctic Alaska to Puget Sound, and on the Asian coast to the Okhotsk Sea, 3–100 fathoms (Rathbun, 1904). Although apparently never abundant enough to support a fishery itself, *P. goniurus* forms a part of shrimp catches of some fishing areas. Hynes (1929) estimated that *P. goniurus* made up about 8% of the shrimp catch in the Petersburg region of Alaska. And, in fact, in Alaskan waters the species has the common name of "humpy" (Schaefers, 1953) because of the prominent carinated lobe of the third abdominal segment. In the San Juan

Islands, Washington, Smith (1937) reported that *P. goniurus* was caught with *P. danae* but fishermen did not distinguish between the two species. Early in 1949 a fishery carried out in Clio Channel, near the northeastern coast of Vancouver Island, produced *P. goniurus* along with the main commercial species, *P. hypsinotus*.

Smith (1937) measured an unspecified number (around 230) of *P. goniurus* taken off Lopez Island, Washington, on March 3, 1937. Most were ovigerous females, centred around 7 cm total length but the rest apparently were unsexed.

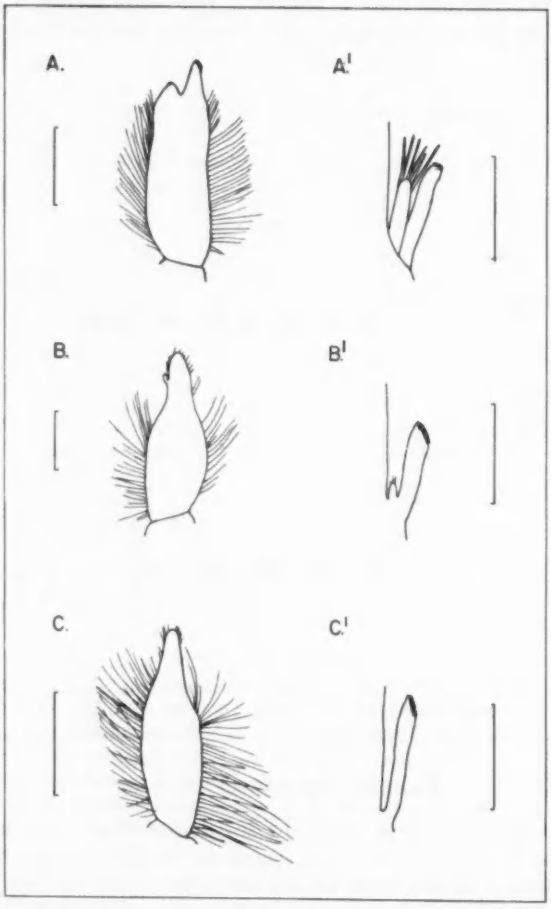

FIG. 27. *Pandalus goniurus*. A—endopodite of first pleopod and A′—portion of endopodite of second pleopod of male with carapace length of 11.0 mm. B, B′—same structures of transitional individual with carapace length of 16.8 mm. C, C′—same structures of female with carapace length of 13.1 mm. Scale represents 1 mm.

On the basis of five specimens, Hynes (1929) found an average egg count of 2000. Apart from the above notes, nothing has been published concerning the biology of this species.

The endopodite of the first pleopod of a male, 11.0 mm, from Pylades Channel (on the east coast of Vancouver Island) on January 29, 1961, is shown in Fig. 27A; at the apex is the copulatory organ armed with cincinnuli. From the same individual the second pleopod's endopodite (Fig. 27A′) had an appendix masculina with six strong, rigid spines at the tip; the appendix interna had a number of cincinnuli scattered about its apex. The transitional phase is represented by the endopodite (Fig. 27B) of the first pleopod of an individual 16.8 mm, collected in Chatham Sound at 51 fathoms on July 27, 1959, which was elongate and had the copulatory organ as a shrivelled appendage with a single remaining cincinnulus. The diagram (Fig. 27B′) of the portion of the endopodite of the second pleopod from the same specimen showed a much reduced appendix masculina with vestiges of several of the spines characteristic of the male phase, and the appendix interna, then enlarged, had several rows of well-formed cincinnuli. The diagram in Fig. 27C, the endopodite of the first pleopod of a female 13.1 mm from Pylades Channel on November 24, 1961, showed the appendage slender and tapering with long setae. The full female condition was found also in the endopodite of the second pleopod from the same specimen (Fig. 27C′) which had only the appendix interna.

Measurements of *P. goniurus* collected from two areas along the east coast of Vancouver Island during 1961 are graphed in Fig. 28. The late January sample (Fig. 28A), from Pylades Channel, 30–35 fathoms, was comprised of 4 males from 10.9 to 11.9 mm; the rest were all ovigerous females, between 11.5 and 13.9 mm. Over 2 months later, the next collection (Fig. 28B) was made in Departure Bay at 20–25 fathoms. Males ranged from 10.0 to 11.4 mm; transition individuals were from 10.9 to 12.6 mm, and the 22 non-ovigerous females were from 10.8 to 13.5 mm. The other sample in this series (Fig. 28C) from Pylades Channel late in November had 11 males ranging from 10.1 to 12.9 mm, and late transitionals from 12.1 to 13.3 mm. Of the 8 females from 12.3 to 14.2 mm, one was ovigerous.

Another sample, taken from a commercial catch made in Clio Channel at 20–30 fathoms early in 1949 and preserved for some years, was measured and is graphed in Fig. 29. Two males measured 12.9 and 13.1 mm, and the females, all ovigerous, ranged from 14.5 to 16.5 mm.

The ages of these male, transitional, and female *P. goniurus* are uncertain. The suggestion is that, in southern British Columbia, growth is rapid and the life span relatively short. In Fig. 28B, males present were at least 1 year old and possibly 2 years, and some individuals of this group were undergoing sex change. Over half a year without other observations had elapsed when the next sample (Fig. 28C) was taken but it is probable that most males, excepting two at 12.9 mm, measured earlier, had completed sex change or were in the transition phase, whereas males between 10 and 12 mm carapace length belong to the 0-group. The latter appear as active males and presumably breed in their first

autumn as in the case of *P. danae*. It is proposed that *P. goniurus* matures and breeds as a male during the first year and then changes sex in the second summer to function as a female at 1½ years; following the hatching of eggs at the end of the second year, it is likely that most females die. Overlapping of length distributions of both sexes indicates that some individuals mature first as females.

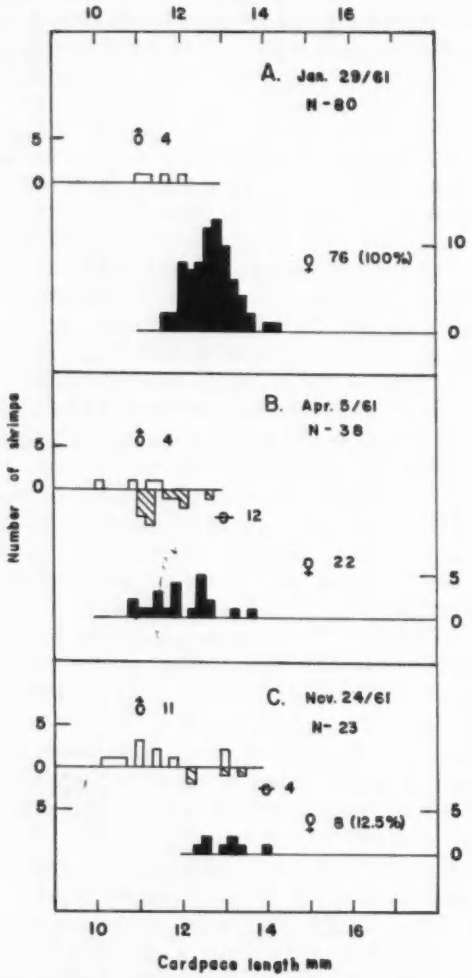

FIG. 28. *Pandalus goniurus*. Carapace length–frequency distributions, east coast of Vancouver Island, January 29 to November 24, 1961. Length data are given in 0.2 mm divisions. Percentage of females that were ovigerous is indicated in parentheses.

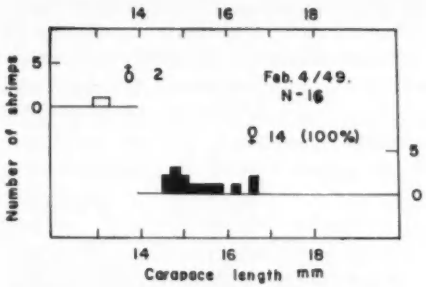

FIG. 29. *Pandalus goniurus*. Carapace length–frequency distributions, Clio Channel, February 4, 1949. Length data are given in 0.2 mm divisions. Percentage of females that were ovigerous is indicated in parentheses.

From the size range of the few specimens examined (Fig. 28B and 29) it is evident that growth is slower on more northern grounds of the province.

The present samples do not fix the ovigerous period exactly but it appears to begin late in November and to be complete by April.

TEMPERATURE AND SALINITY OBSERVATIONS

The maximum and minimum bottom temperatures and salinities observed during the present study in relation to the occurrence of each of seven pandalid species are tabulated in Tables IX and X, respectively. Most of these maxima and minima were found in Indian Arm and Burrard Inlet.

TABLE IX. Maximum and minimum bottom temperatures related to occurrence of seven pandalid shrimps.

Species	Temperature (°C)	Depth (m)	Date
Pandalopsis dispar	10.50	95	Aug. 28/61
	7.61	73	Jan. 15/62
Pandalus jordani	10.97	60	Aug. 28/61
	7.44	40	Jan. 29/61
P. borealis	11.08	60	Aug. 24/61
	7.37	45	Jan. 16/61
P. platyceros	10.97	60	Aug. 28/61
	7.61	27	Jan. 18/62
P. danae	12.32	54	Aug. 24/61
	7.37	45	Jan. 16/62
P. hypsinotus	11.08	60	Aug. 24/62
	7.44	40	Jan. 29/61
P. montagui tridens	9.53	91	Nov. 7/61
	8.56	154	Apr. 7/61

TABLE X. Maximum and minimum bottom salinities related to occurrence of seven pandalid species.

Species	Salinity (‰)	Depth (*m*)	Date
Pandalopsis dispar	30.84	146	Oct. 27/61
	26.73	109	Aug. 24/61
Pandalus jordani	30.83	128	Dec. 1/60
	28.69	36	Mar. 29/61
P. borealis	30.84	146	Oct. 27/61
	25.88	54	June 7/61
P. platyceros	30.83	128	Dec. 1/60
	26.42	64	Oct. 25/61
P. danae	30.06	45	Jan. 16/61
	25.23	54	June 7/61
P. hypsinotus	30.56	92	Nov. 7/61
	25.88	54	June 7/61
P. montagui tridens	30.54	91	Nov. 7/61
	29.24	73	Aug. 31/61

Elsewhere, values have been reported for three species and a subspecies, considered here. Allen (1959) stated that adult *P. borealis* occur within the temperature range −1.68 to 11.13°C. According to him, this species is rarely found in European waters with salinities less than 34‰; this contrasts with the picture on British Columbia grounds where *P. borealis* normally lives in salinities of 30‰ or less. *Pandalus jordani* has been collected outside San Francisco Bay at depths exceeding 29 fathoms where temperatures were from 9.3 to 12.2°C and salinities from 33.9 to 34.4‰ (Schmitt, 1921). Thus, the temperature and salinity ranges found in the Strait of Georgia for *P. jordani* are lower than the range observed near San Francisco. In the same paper, Schmitt stated that inside San Francisco Bay, *P. danae* did not occur at stations with temperatures higher than 12.2°C and salinities less than 25.7‰; these limits are exceeded slightly in the values in Tables IX and X for the same species, observed in Burrard Inlet. Although Mistakidis (1957) does not express upper and lower limits for *P. montagui* along the east coast of England, I have selected from his hydrographic records, accompanying various shrimp catches, values from 0.45 to 20.15°C, and from 24.81 to 35.30‰ for temperatures and salinities, respectively. Thus, temperature and salinity observations at localities in British Columbia waters where *P. montagui tridens* occurred are well within the ranges known for the European form, *P. montagui*.

DISCUSSION

GROWTH

A summary of the growth of six British Columbia species and that of *Pandalus borealis* in Oslo Fjord, taken from Rasmussen (1953), is shown in Fig. 30. Except for *P. borealis* and *P. jordani*, local species exceed in size, at comparable

ages, *P. borealis* in Norwegian waters. *P. platyceros* exceeds all others in body length and weight at any age. In the first year, from August to April, young male prawns more than triple their body weight, and in the second year the increase is about 155% (Table VI). *Pandalus hypsinotus*, at least for the first 2 years, is next to the prawn in growth rate, with a similar relative increase in body weight (Table VII), but thereafter the rate decreases. *Pandalus danae* is somewhat greater in size than *P. borealis* or *P. jordani* during the first year, but subsequently the difference lessens.

Three of the species, *P. platyceros*, *P. hypsinotus*, and *P. danae*, which exhibit rapid growth (Fig. 30) during the first year, have in common the habitation of shallow-water bays and inlets. It is suggested that this habitat in the late summer and autumn is a favourable one, because detrital material remaining after summer production of plankton and large algae, and various scavenging

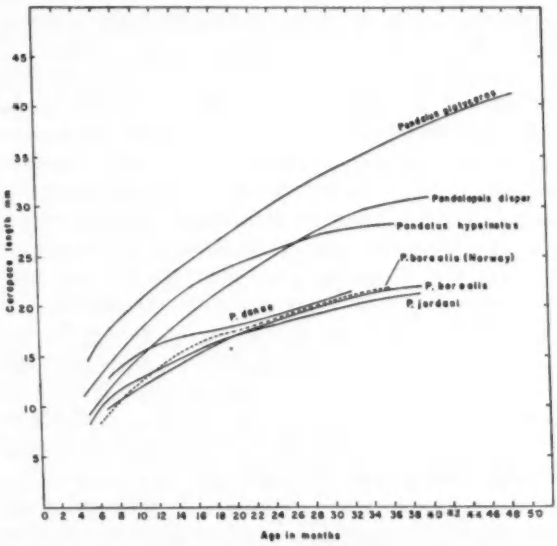

FIG. 30. Average growth curves of six species of British Columbia pandalid shrimps and *Pandalus borealis* in Oslo Fjord.

organisms such as amphipods and mysids, appear to be a rich source of food. Young *P. platyceros* migrate to deeper water late in the first year and evidently adapt to feeding on the epifauna of rocky bottom and on the detritus in mud to sustain the continuing rapid growth. Unlike the prawn, *P. hypsinotus* and *P. danae* do not normally leave the shallower region where the food supply apparently is adequate for function and growth during the rest of their relatively shorter lives.

Pandalopsis dispar grows fairly steadily throughout its life and ultimately is second to the prawn in length and weight. Abundant populations of the

juveniles and adults of this species inhabit the same grounds, usually in the deeper sections of inlets and bays. There, organic detritus, arising from plankton production in upper layers or from land run-off, settles and collects to form a rich source of food for *P. dispar* at all post-larval stages. Exploratory fishing on open coastal grounds (Alverson *et al.*, 1960) has yielded this shrimp in only small numbers because there, apparently, through wide dispersal, detritus is not concentrated sufficiently for *P. dispar* to flourish.

Reproduction

Three other Pacific pandalid shrimps, *P. montagui tridens*, *P. stenolepis*, and *P. goniurus*, are now known to be hermaphroditic. The known bisexual species of *Pandalus* are *P. bonnieri* (Pike, 1952) and *P. propinquus* (Jagersten, 1936). There is some evidence that the sexes are separate in *Dichelopandalus leptocerus* (Smith) (Scattergood, 1952). All the three latter species occur in the Atlantic, although *D. leptocerus* has been recorded once from the Pacific, off Shumagin Bank (Rathbun, 1904).

As shown earlier, nine pandalid shrimps occurring in British Columbia waters are hermaphroditic. Some species exhibit practically full hermaphroditism, that is, all individuals pass through both male and female phases; other species, along with their normal hermaphroditism, have individuals which function only as females. It appears worthwhile to consider this phenomenon with regard to geographic and bathymetric distributions. Species dominantly hermaphroditic include *Pandalopsis dispar* and *Pandalus platyceros*, and likely *P. montagui tridens* and *P. stenolepis*. These four species have in common fairly extensive ranges with southern limits south of the British Columbia coast. *Pandalopsis dispar*, which does not extend as far southward as the others, is similar to the prawn in that all or most of its life is spent in deeper water. Off California near its southern limit the prawn apparently does not have early maturing of females (W. A. Dahlstrom, unpublished). Nothing is known of the degree of hermaphroditism of *Pandalus stenolepis* and *P. montagui tridens* in sectors of their ranges south of British Columbia.

Samples of the European form, *P. montagui*, taken off the English coast, presumably near the southern limit of its range, showed a relatively high proportion, 11–44%, of primary females (Mistakidis, 1957). Yet, essentially the same ratios were reported by Jagersten (1936) along the west coast of Sweden. According to Squires (1961) no small females of *P. montagui* occurred in samples from the Newfoundland coast, approximately midway in its distribution from Greenland to Rhode Island in the western Atlantic.

Three shrimps which exhibit partial hermaphroditism, *P. borealis*, *P. hypsinotus*, and *P. goniurus*, reach their southern limits off or near the southern British Columbia coast. Although primary females of *P. borealis* were not apparent in present samples, females were estimated to be about 47% of the mature second-year individuals. Allen (1959) found for *P. borealis* in the North Sea (the southern limit of its range in European waters) that about 35% of the population consisted of primary and secondary females. Hermaphroditism is

more pronounced in this species along the Norwegian coast where Rasmussen's data (1953) showed only 5–10% of early maturing females. Within the western Atlantic range of *P. borealis*, however, the published data are inconclusive; Squires (1961) found no primary females around Newfoundland, and among a collection of 228 females measured by Scattergood (1952) off Maine, one which fell within the male size range was present. In present *P. hypsinotus* samples, both primary (8–21%) and secondary females appeared. Although Igarashi (1951) writes of a fully hermaphroditic condition in this species from Hokkaido, Japan, his tabulated measurements suggest that early maturing females do occur. The limited sampling of *P. goniurus* here has not given an estimate of primary, if present, and secondary females, and information on sex ratios in other parts of the range is unavailable.

Pandalus jordani is another species found during the present study to deviate from the normal hermaphroditic pattern. Primary females were not identified but the proportion of mature females in the second-year group, though the estimate was less certain, was over 40%, like *P. borealis*. The recorded northern limit of *P. jordani* is in Alaskan waters but this shrimp has not been found in quantity north of British Columbia (Ronholt, 1963). The significance of this with respect to reproduction is that Vancouver Island populations, in the region where *P. jordani* apparently ceases to flourish, show a higher proportion of early maturing females than evidently occur off Oregon.

The remaining species to consider is *P. danae*. Because of the difficulty in aging this shrimp the ratio of secondary females is uncertain but likely is high. Present samples were collected in the northern half of its range and in the shallower part of the bathymetric distribution.

Thus, there is evidence to indicate that hermaphroditism is more dominan among pandalids living in the optimum sections of their geographic ranges or in deeper-water species, and that the occurrence of non-hermaphroditic females is associated with populations of species near the limits of the geographic ranges or in shallower water. A suggested explanation is that at the extremes of the ranges or in shallow water, shrimps encounter adverse environmental conditions, and under these stresses there is a tendency for early appearance of the female phase. A hormonal control of sex is accepted by Allen (1959), and he suggests that temperature will influence the onset of maturity. If so, other external factors such as salinity, substratum, and competition may also exert an effect on sex determination. And, as pointed out earlier in comments on temperature and salinity observations, three of the partly hermaphroditic species, *P. jordani*, *P. borealis*, and *P. danae*, occur off British Columbia in colder and less saline water than elsewhere in their ranges. *Pandalus montagui tridens*, assumed to follow the normal hermaphroditic pattern around Vancouver Island, was found within narrower ranges of temperature and salinity than the European *P. montagui* which has primary females.

The significance of the presence of non-hermaphroditic females is that by maturing a year earlier these individuals increase the reproductive potential of shrimp populations. Because of their smaller size, second-year females, of

course, produce fewer eggs than older and larger hermaphroditic females. This may not be a disadvantage because, as Birch (1948) has shown for insects, production of eggs early in life, though in relatively fewer numbers, largely determines the population parameter "intrinsic rate of increase". This effect is less pronounced in shrimps because the length of a generation is considerably longer. Birch has suggested that for a species to survive in a particular environment a minimum value for the intrinsic rate of natural increase may have to be evolved. In shrimp populations living at the extremes of their ranges the presence of early females is likely a mechanism for keeping the rate of increase sufficiently high to balance the effects of potentially unfavourable environments. Natural selection may operate to provide such populations with a high proportion of individuals which mature directly as females, perhaps requiring the stimulus of adverse conditions. If such is the case, these shrimp species could be expected to maintain themselves with varying conditions; if non-hermaphroditic females occur independently of external factors then at times the population presumably would increase. The question is complex and for elucidation requires extensive sampling throughout the ranges of the pandalids concerned.

SUMMARY

1. Information is presented on the ranges, habitats, and commercial exploitation of nine British Columbia shrimps of the family Pandalidae. A key for their identification is also included.

2. Over 27,400 specimens of *Pandalopsis dispar*, *Pandalus jordani*, *P. borealis*, *P. platyceros*, *P. hypsinotus*, *P. danae*, *P. montagui tridens*, *P. stenolepis*, and *P. goniurus* were measured, and sexed, and the reproductive condition determined, mostly from grounds adjacent to the Strait of Georgia during November 1960 to October 1962.

3. A total of 1291 specimens of six species was measured to determine the ratio of carapace length to total weight; the carapace length–weight relationships of six species were determined, based on 1694 individuals.

4. Growth rates and ages were estimated; the mean carapace and total lengths, and mean total body weights at yearly or more frequent intervals, are tabulated for six species, *Pandalopsis dispar*, *Pandalus jordani*, *P. borealis*, *P. platyceros*, *P. hypsinotus*, and *P. danae*.

5. All of the above six species are normally hermaphroditic but in four, *P. jordani*, *P. borealis*, *P. hypsinotus*, and *P. danae*, varying numbers of early maturing females occur.

6. Three pandalid shrimps, *P. montagui tridens*, *P. stenolepis*, and *P. goniurus*, are shown for the first time to undergo sex change. Knowledge of the biology of these species is scanty.

7. Maximum and minimum bottom temperatures and salinities as observed for seven species are tabulated.

8. Growth rate as related to habitat and food supply is discussed, and some remarks are made concerning reproduction and geographic and bathymetric ranges.

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REFERENCES

ALLEN, J. A. 1959. On the biology of *Pandalus borealis* Krøyer, with reference to a population off the Northumberland coast. *J. Mar. Biol. Assoc. U.K.*, **38**(1): 189–220.

ALVERSON, D. L., R. L. MCNEELY AND H. C. JOHNSON. 1960. Results of exploratory shrimp fishing off Washington and Oregon (1958). *Comm. Fish. Rev.*, **22**(1): 1–11.

BERKELEY, A. A. 1929a. Sex reversal in *Pandalus danae*. *Am. Nat.*, **63**: 571–573.

1929b. A study of the shrimps of British Columbia. *Biol. Bd. Canada, Pacific Prog. Rept.*, No. 4, pp. 9–10.

1930. The post-embryonic development of the common pandalids of British Columbia. *Contrib. Canadian Biol., N.S.*, **6**(6): 79–163.

BIRCH, L. C. 1948. The intrinsic rate of natural increase of an insect population. *J. Anim. Ecol.*, **17**(1): 15–26.

BUTLER, T. H. 1959. Results of shrimp trawling by *Investigator No. 1*, June 1959. *Fish. Res. Bd. Canada, Nanaimo Biological Station Circular*, No. 55, 7 pp.

1963. An improved prawn trap. *Ibid.*, No. 67, 7 pp.

GOODWIN, D. G. 1952. Crustacea collected during the 1950 bottomfish investigations of the M. V. *N. B. Scofield*. *California Fish and Game*, **38**(2): 163–181.

GREENWOOD, M. R. 1959. Shrimp exploration in central Alaskan waters by M/V *John N. Cobb*, July–August 1958. *Comm. Fish. Rev.*, **21**(7): 1–19.

HJORT, J., AND J. T. RUUD. 1938. Deep-sea prawn fisheries and their problems. *Hvalrad. Skr.*, No. 17, 144 pp.

HYNES, F. W. 1930. Shrimp fishery of southeast Alaska. Appendix to the Report of the U.S. Commissioner of Fisheries for 1929. B. F. Doc. No. 1052, Appendix 1, 18 pp.

IGARASHI, T. 1951. Studies on *Pandalus hypsinotus* Brandt in Funka Bay, Hokkaido. *Bull. Fac. Fish., Hokkaido Univ.*, **2**(1): 1–9.

JAGERSTEN, G. 1936. Uber die Gesclechts-verhaltnisse und das Wachtum bei *Pandalus*. *Ark. Zool.*, 28, A(20): 1–26.

MISTAKIDIS, M. N. 1957. The biology of *Pandalus montagui* Leach. *Ministry of Agriculture, Fisheries and Food, London, Fishery Invest.*, Ser. 2, **21**(4): 52 pp.

NEEDLER, A. B. 1938. The larval development of *Pandalus stenolepis*. *J. Fish. Res. Bd. Canada*, **4**(2): 88–95.

PIKE, R. B. 1952. Notes on the biology of the prawn *Pandalus bonnieri* Caullery. *J. Mar. Biol. Assoc. U.K.*, **31**(2): 259–267.

PRUTER, A. T., AND G. Y. HARRY. 1952. Results of preliminary shrimp explorations off the Oregon coast. *Fish. Comm. Oregon Res. Briefs*, **4**(1): 1–13.

RASMUSSEN, B. 1953. On the geographical variation in growth and sexual development of the deep sea prawn (*Pandalus borealis* Kr.). *Rept. Norwegian Fish. Mar. Invest.*, **10**(3): 160 pp.

RATHBUN, M. J. 1904. Decapod crustaceans of the northwest coast of North America. *Harriman Alaska Exped. Ser.*, **10**: 210 pp.

RONHOLT, L. L. 1963. Distribution and relative abundance of commercially important pandalid shrimps in the northeastern Pacific Ocean. *U.S. Fish Wildlife Serv., Spec. Sci. Rep., Fisheries*, No. 449, 28 pp.

RONHOLT, L. L., AND A. R. MAGILL. 1961. Biological observations and results of the 1960 *John N. Cobb* exploratory shrimp cruise off the central Oregon coast. *Fish. Comm. Oregon Res. Briefs*, **8**(1): 31–46.

SCATTERGOOD, L. W. 1952. The northern shrimp fishery of Maine. *Comm. Fish. Rev.*, **14**(1): 1–15.

SCHAEFERS, E. A. 1953. Shellfish explorations in certain southeastern Alaskan waters by the *John N. Cobb*, spring 1952. *Comm. Fish. Rev.*, **15**(3): 1–18.

SCHAEFERS, E. A., AND H. C. JOHNSON. 1957. Shrimp explorations off the Washington coast, fall 1955 and spring 1956. *Comm. Fish. Rev.*, **19**(1): 9–25.

SCHMITT, W. L. 1921. The marine decapod Crustacea of California. *Univ. Calif. Publ. Zool.*, No. 23, 470 pp.

SMITH, R. T. 1937. Observations on the shrimp fishery in Puget Sound. *Washington Dept. Fish. Biol. Rept.*, No. 36D, 29 pp.

SQUIRES, H. J. 1961. Shrimp survey in the Newfoundland fishing area, 1957 and 1958. *Bull. Fish. Res. Bd. Canada*, No. 129, 29 pp.

TEGELBERG, H. C., AND J. M. SMITH. 1957. Observations on the distribution and biology of the pink shrimp (*Pandalus jordani*) off the Washington coast. *Washington Dept. Fish Res. Papers*, **2**(1): 1–10.

Illumination, Vision, and Schooling of *Astyanax mexicanus* (Fillipi)[1,2]

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ABSTRACT

The schooling behavior of both light- and dark-adapted *Astyanax mexicanus* (Fillipi) was observed under sudden changes in illumination in the range 10^{-7} to 10^{0} ft-c at different times in the diel cycle. In darkness the fish were dispersed, swam slowly, and showed no signs of mutual recognition of one another. There was no simple relationship between the schooling response and the illumination, but in general the frequency, size, and durability of schools were proportional to the illumination. Schooling was maximal above 10^{-1} ft-c and approached zero at 10^{-6} ft-c. The schooling at any level of illumination varied with the testing procedures. The lowest responses were obtained with a gradual reduction of illumination in the evening. At illumination below 10^{-2} ft-c maximal schooling was obtained from dark-adapted fish. From 10^{-2} ft-c upward, it was obtained from light-adapted fish. The schooling rate (number of fish in a school) reached 100% for a few seconds at 10^{-3} ft-c and for longer periods at higher illumination, but was never sustained at the 100% level throughout any 4-min period. Schooling was generally enhanced by any sudden change in illumination but was never sustained at a high rate under monotonous conditions. The decline with monotony was equated with reduction of the fright situation. The decline with reduced illumination was attributed to the reduction of visual acuity and visibility, and occurred at a greater rate among faster-swimming fish. *Notemigonus crysoleucas* (Mitchill), swimming very slowly, exhibited high schooling rates at 10^{-5} ft-c. Earlier work showed that the retina of *Astyanax* in continuous darkness undergoes a rhythmic diel shift. The behavior showed that this retinal shift is not the classical transformation from scotopic to photopic vision. Dark-adapted during the day, the fish demonstrated rapid visual perception under sudden illumination at any value from 10^{-6} to $10^{-0.69}$ ft-c. Dark-adapted fish at night exhibited increasingly longer light-shock reactions with sudden illumination above 10^{-2} ft-c. According to schooling responses the cone threshold was about 10^{-3} ft-c, and the rod threshold about 10^{-6} ft-c. The mesopic range extended from about 10^{-3} to 10^{-2} ft-c.

INTRODUCTION

One of the central problems in the studies of schooling behaviour of fishes has been to determine which sense organs are involved in keeping the school intact and co-ordinated. The early efforts, supported by growing evidence, demonstrated that vision is of primary importance. Breder (1959), in summary of the literature, concluded that vision is essential for schooling; without vision, regardless of the other sense organs, fish do not school. (The terminology for groups of fishes will follow that used by Breder.)

Atz (1953) in his thorough review of the subject pointed out that non-visual sensory modalities — auditory, acoustico-lateralis, and chemoreceptive — are

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thought to play some part in the co-ordination of a school, but, because vision plays such a dominant role, these relations remain unclear. He concluded that an aggregation, however, may be co-ordinated by non-visual information.

Moulton (1960) reported that a blinded individual, *Anchoviella choerostoma*, joined a school of normal individuals whenever the school streamed and veered and that it was presumably responding to the swimming sounds. Although this was an unnatural situation because the majority of the fish were normal, Moulton interpreted the response to indicate that auditory communication may become more important to the integrity of a group as illumination decreases. Keenleyside (1955) noting that blinded *Scardinius erythrophthalmus* failed to school but remained in an area where odors of conspecific individuals were detected suggested that olfaction might at least prevent groups from scattering widely at night.

While the question of relative involvement of the non-visual sense organs remains unsettled, the role of vision in schooling has been actively pursued in the laboratory. Several studies have focused on the question of schooling in relation to illumination. A tabulation by Shaw (1961) of published results showed schools dispersing at different values of illumination ranging from about 10^{-4} to 3 ft-c depending on the species.

In my initial studies on *Astyanax mexicanus* (unpublished paper read before the American Society of Limnology and Oceanography at the meetings of the American Institute of Biological Sciences, 1961), it was established that the minimum illumination under which schooling occurred varied with the experimental procedures. The lowest responses were obtained by either gradual increases or decreases in illumination. However, owing to inadequate equipment, I was uncertain of the accuracy of some of the measurements, and it seemed wise to repeat the experiments with improved equipment in order to solidify the conclusions. Using the refined equipment described in this paper, experiments were performed to determine the schooling behaviour under varying illumination.

APPARATUS

Although the apparatus will accommodate a variety of behavioral studies and is not restricted by any range of illumination, it was designed specifically to observe continuously from darkness through any level of illumination and is thus a design restricted to the limitations of an infrared image converter.

The facilities consisted of a first floor aquarium room which could be made light-tight, and a second floor observation room located directly over the center of the first room.

The drawing in Fig. 1 shows the major features of the apparatus. The aquarium, constructed of polyethylene $\frac{1}{8}$ inch thick, was 6 ft in diameter and 1 ft deep, with a flat bottom. It rested on a sheet of clear plexiglas ($\frac{1}{2}$ inch thick) which in turn rested on a horizontal bed of steel channel beams ($\frac{3}{8}$ inch edge) spaced 1 ft apart and 2 ft off the floor. The fish were confined by a circular wall of clear plexiglas, 4 ft in diameter, centered in the polyethylene aquarium. This confinement was necessary because the fish could not be seen easily against the polyethylene walls.

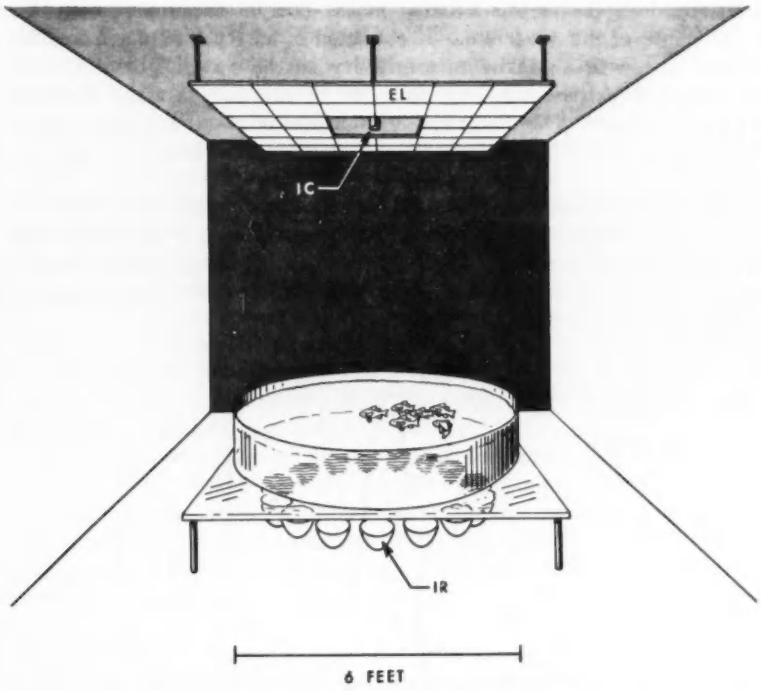

FIG. 1. A diagrammatic view of the major components in the experimental apparatus: EL = electroluminescent panels, IC = objective end of infrared image-converter, IR = infrared lamps. The infrared lamps in this drawing, arranged in a circle beneath the aquarium, are individual metal housings equipped with military surplus infrared filters. This is an alternate design. The infrared sources used in the present experiments were rectangular boxes with black carrara glass as a filter.

Infrared sources were distributed on the floor to project radiation up through the bottom of the aquarium. A sheet of drawing paper suspended over the infrared sources diffused the radiation so that in the image, the fish appeared as dark silhouettes against the light-colored bottom. The output of the infrared sources was controlled through a variable transformer to obtain the minimum useful level of radiation and also to eliminate the spark noise that would accompany the sudden switching of a large load to the line supply. The infrared radiation was obtained by filtering tungsten emission through black carrara glass (Pittsburg Plate Glass Co.). The entire source consisted of 10 contiguous rectangular boxes, 10 × 12 × 36 inches, each housing four red bulbs (25-w tungsten) and faced with a plate of $\frac{5}{8}$ inch black carrara glass. Since this glass transmits visible radiation in the blue region of the spectrum, the bulbs had to be operated at about $\frac{2}{3}$ rated voltage in order to reduce the visible component below the level to which the fish responded.

The infrared viewer was situated in the floor of the upper room directly over the center of the aquarium. It consisted of an RCA 6914 infrared image-converter tube, with a maximum sensitivity at the wavelength of 8000 Å; an f 1.3, 15-mm objective lens (Angénieux for 16-mm camera); and a simple 10-power eyepiece lens. The image had a brightness of about $\frac{1}{100}$ ft-lamberts which made it visible to the light-adapted eye, but it was not bright enough to photograph.

The source of visible radiation was green electroluminescent panels (RCA) which had the average emission spectrum shown in Fig. 2. Each panel was 1 ft square including an inactive margin $\frac{1}{4}$ inch wide. In application, the source consisted of 32 panels mounted contiguously on the ceiling 8 ft above the aquarium over an area 6 × 6 ft minus a central area, 2 × 2 ft.

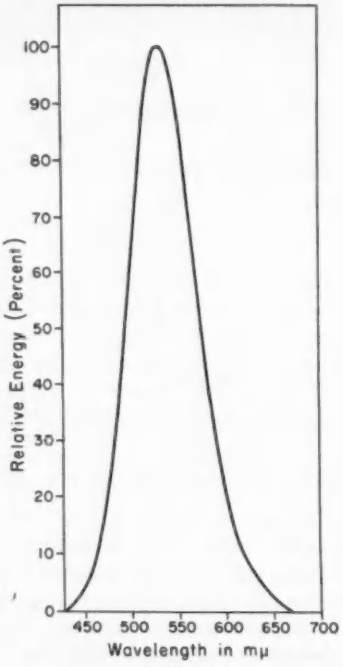

FIG. 2. Spectral emission of green electroluminescent panels.

Electroluminescent panels were used because the emission spectrum is independent of voltage so the quality of illumination remains constant throughout the range of brightness which in turn can be varied smoothly through controlled voltage. Because the panels emit little energy in the infrared region, the reflected light from the surface of the water was scarcely detected through the image-converter. The surface glare from a tungsten source, on the other hand, is quite distracting.

The photometer assembly was a Hewlett-Packard microvolt ammeter 425 A; a Northeast Scientific Corporation regulated high voltage supply, RE-5002; and 3 RCA 8053 multiplier phototubes in water-tight encasements. These phototubes had an S-11 spectral response (see Engstrom, 1960). The photometer was calibrated in amps/ft-c of radiant flux from a calibrated tungsten source at a color temperature of 2870°K. The response of the photometer to green panels in amps/ft-c was determined by calculation.

In order to maintain a tolerable anode current and make linear measurements of light from the level of minimum response of the phototube, which was determined by the dark current, to a maximum of 10^{1} ft-c, the windows of the phototubes were partly masked and the tubes were operated at different voltages. One tube with an aperture 1 inch in diameter, operating at 1000 v, measured the light from 10^{-8} through 10^{-3} ft-c. The other tube with a $\frac{1}{16}$-inch aperture operating at 1000 v, measured from 10^{-4} through 10^{-2} ft-c, and at 750 v, from 10^{-2} through 10 ft-c. The third tube, with appropriate masks, was used as a check against the other two.

The accuracy of the ammeter was 3% of full scale. An additional error, ranging as high as 10%, stemmed from the observer's inability to reproduce interpolated readings on the ammeter scale. A final additional error of about 2% was attributed to the non-uniform directional response of the phototube. Since the panels were not of identical brightness, the reading of the photometer varied as the phototube was rotated about its vertical axis. The total known photometric error was in the order of ±15%.

The distribution of downward illumination at the periphery within the plexiglas confinement, measured $\frac{1}{4}$ inch below the surface of the water, differed irregularly from the value at the center, and by different amounts at different orders of brightness. At 10^{-4} ft-c, it varied from 87.5 to 95.5%; at 10^{0} ft-c, which gave the worst distribution, it varied from 81 to 101%. At a given light output, the downward illumination $\frac{1}{4}$ inch below the surface of the water was about 23% greater than that $\frac{1}{4}$ inch above the surface. Considering Snell's Law, the light entering the phototube below water would be about 2% greater than that $\frac{1}{4}$ inch above the surface. The increase in light below water (21%) was derived from internal reflection from the glossy surfaces and the "white" color of the aquarium. The horizontal illumination at the center of the aquarium was 33% of the downward illumination above the surface. The useful illumination for the fish was unknown, but was somewhere in the range ±25% to −33% of the amount striking the surface of the water. The photometric values reported in this paper are of the downward illumination at the center of the aquarium measured $\frac{1}{4}$ inch above the surface of the water. The readings from the central phototube were correlated with those from tubes standing around the outside periphery of the aquarium and, during experiments, the light was measured by the peripheral phototubes.

METHODS

There were no objects in the aquarium and the water was still, without circulation or aeration. The aquarium was surrounded by black curtains which

hung at the periphery of the light source from ceiling to floor. The curtains standardized the surroundings and minimized reflections of visible light. A tungsten lamp at the ceiling approximately over the center of the aquarium was the source for maintaining an artificial diel cycle of $9\frac{3}{4}$ hr light, $14\frac{1}{4}$ hr darkness through an electric clock switch. Illumination from the lamp, at the surface of the water, was about $10^{0.69}$ ft-c. The noise of the switch, going on or off, was clearly audible in the aquarium room. Two 1400-w air conditioners operated continuously, one in the aquarium room and one in the overhead observation room. This general steady background noise was punctuated by occasional transient noises of aircraft and the starting or stopping of air conditioning motors in the building. The temperature of the water throughout the studies was 25±1°C.

Six adult *Astyanax* (total length 75±5 mm) were put into the aquarium April 12, 1963. They had been maintained along with 12 others in the laboratory for over 1 year in a large aquarium furnished with plants, shelters and aerator. They were subjected to more or less continuous testing as suitable procedures were developed. The tests reported in this paper were staged July 5–25, 1963.

The standard observation period was 4 min, beginning at the instant of changing the illumination, but was extended to 8 or 9 min when the dark-adapted fish at night were exposed to the higher levels of illumination. The behaviour of the fish was reported continuously to a recorder who co-ordinated the events with time. The observer reported primarily on the numbers of fish in schools and attempted to report any change at the instant it occurred. The fish were considered schooled when they swam in the same direction and the distance between individuals was less than about 4 times their length. To compare schooling activities, the schools were assigned index numbers as given in Table I. At other points in the paper reference is made to the "schooling rate". This is the area within a histogram divided by the total area of the graph, expressed as a percentage. The maximum rate, 100%, would be the product of a schooling index of 10 times 240 sec.

TABLE I. Indices used for comparison of schooling responses. Note that the indices are weighted in favor of a single larger school against two or more smaller simultaneous schools although the total numbers of fish in the smaller schools might exceed that of the single school.

Schooling index	No. of schools	No. of fish in schools
1	1	2
2	2	2, 2
3	3	2, 2, 2
4	1	3
5	2	3, 2
6	2	3, 3
7	1	4
8	2	4, 2
9	1	5
10	1	6

General information on the behaviour of *Astyanax* in the laboratory was accumulated throughout the course of their confinement and included many observations apart from the specific series of tests, which are described below:

SERIES A. July 5 to 20. Dark-adapted fish. One observation each morning. The fish were observed for 1 min in darkness. The cycle lamp was disconnected and in its place, at the instant the electrical clock switch went on (0845 hr), the panels were turned on at a particular illumination randomly prescribed. The values of illumination ranged in logarithmic units from 10^{-7} to 10^{0} ft-c.

SERIES B. July 5 to 20. Dark-adapted fish. One observation each night at about 2030 hr. The fish were in darkness from 1830 hr. They were observed for 1 min in darkness, then the panels were turned on instantly at a randomly prescribed illumination. The range of values was the same as in Series A.

SERIES C. July 21. Light-adapted fish. Tungsten light turned on, in cycle, at 0845 hr. Series began at 1000 hr. To prepare for tests at each level of illumination, the tungsten light was turned off for 1 min. Then the testing light was turned on. At the end of each test the tungsten light was turned on for 1 min and was again turned off for 1 min preceding each test. The testing values of illumination for each 4-min observation, in the order given, were $10^{0.69}$ ft-c (tungsten); 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} ft-c (panels).

SERIES D. July 21. Light-adapted fish. The series began at 1820 hr with a 4-min record of the behavior of the fish under the tungsten light. At 1830 hr, the instant the tungsten light was turned off by the clock switch, the panels were turned on at an illumination of 10^{-1} ft-c. The illumination was kept at this value for 15 min and then was instantly reduced by 1 log unit, kept there for 15 min and then instantly reduçed by 1 log unit, and so on to the end of the series. The behaviour of the fish was recorded during the first and last 4 min of each 15-min interval.

Cursory observations were made on 7 adult golden shiners, *Notemigonus crysoleucus* (Mitchill), (average length, 130 mm) on May 16. They had been maintained in the laboratory in a 20-gal, 1 × 1 × 2½-ft aquarium for 1 week. They were transferred to the testing aquarium in darkness at 1450 hr and observations began at 1630 hr. The fish were exposed to sudden illumination at logarithmic increments beginning at 10^{-7} ft-c, with 15-min periods of darkness between tests. They were subjected to the same tests again at 1945 hr the same day, having been in darkness from 1720 hr.

RESULTS

In its natural habitats *Astyanax* swims in schools during the day and retires to quiet water with approaching sunset to rest down on the vegetation. The level of illumination which induces resting is not known.

In the laboratory *Astyanax* was generally pugnacious. In a 20-gal aquarium the fighting was usually intense and after a few days there were few survivors. In a circular 50-gal aquarium, 4 ft in diameter and 8 inches in depth, the fighting decreased markedly, but periodically an individual was killed. Mature fish were

more pugnacious than juveniles. The latter expressed hierarchical attitudes, but were not destructive until they reached a total length of about 30 mm. The intensity of fighting appeared to correlate most conspicuously with the dimensions of the confinement and not with the fish to volume ratio. The fighting among 8 fish in a 20-gal rectangular aquarium was far greater than among 60 fish in a 50-gal circular aquarium. In part, the fighting depended on the nature of the individual fish and the occasional group fared well in a 20-gal aquarium.

In the experimental aquarium under the artificial diel cycle the behavior of the fish changed with time. For about the first 2 weeks it was impractical to test their responses to low levels of illumination because in darkness as well as in subdued light they swam rapidly and independently around the periphery of the aquarium. Individuals swimming in opposite directions continued by one another with no apparent response. Under the tungsten light the fish existed in a compact school generally near the periphery of the aquarium. When the curtains were opened in order to feed the fish the school moved to the far side of the aquarium and remained relatively motionless and never came over to feed in the presence of the observer.

By the end of the first 2 weeks the fish had adjusted to the new situation. In the mornings they formed a relatively compact school within 15–20 sec after the tungsten cycle-light was turned on, but soon relaxed and during the day fluctuated from a wide distribution to a school. They formed a compact school in response to an unusual noise or the appearance of a person beside the aquarium.

At night in darkness the fish were always widely scattered and relatively calm. Three or four fish were generally dispersed across the area of the aquarium and moved very slowly. The others generally swam in isolation in complete circuits around the periphery at a rate of about 6 inches/sec, but there was no single persistent distribution. Fish frequently encountered one another and often changed course abruptly to avoid collisions, but did not attempt to follow one another. There was one notable exception. Each morning, in the darkness just preceding the switching on of the tungsten light, the fish became increasingly more active; they swam rapidly (1–1½ ft/sec), and predominantly around the periphery of the aquarium. This behavior was probably one manifestation of the daily rhythm which was described by John and Haut (1964).

The behavior of the fish in darkness during the daytime part of the cycle was the same as at night. Throughout the experiments the fish swam predominantly around the periphery of the aquarium. At times they lapsed into escape behavior and swam with their snouts pressed against the plexiglas wall. This behavior was at its maximum during the first days following the introduction of the fish into the aquarium and although it declined, it never ceased entirely.

During either day or night, the minimum illumination that induced a response from the dark-adapted fish was about 10^{-7} ft-c. The response was entirely photokinetic with no schooling and was difficult to detect because it was a weak change either in the rate of swimming or in the areal distribution

of the fish. It probably represented response to the overhead lights rather than to illumination.

The minimum illumination under which the fish schooled and the intensity of schooling varied with the testing procedure. In Series A (Fig. 3) the minimum light for schooling was 10^{-6} ft-c. As two slow-moving fish approached each other they formed a pinwheel, each fish circling tightly in pursuit of the other. The pinwheels never lasted more than 3 sec, nor involved more than two fish, and only occurred in about 20% of the cases of two slowly converging fish. The pinwheel dissolved quietly or the two fish suddenly parted as though they became alarmed. At 10^{-5} ft-c, schools formed more frequently and the members generally swam together side by side or in file, but sometimes formed a pinwheel. The durability of the schools varied with the rates of swimming. Frequently one fish briefly pursued another but failed to establish a school as the leader darted away. As they had at 10^{-6} ft-c, the schools at 10^{-5} ft-c sometimes disintegrated suddenly as though the fish became alarmed. At progressively higher

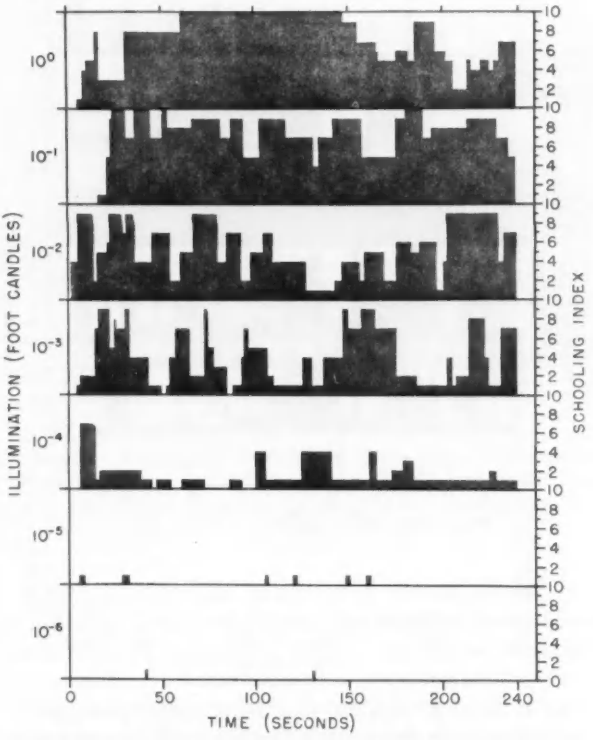

FIG. 3. *Series A.* Schooling responses of dark-adapted *Astyanax* under green light which was turned on at a randomly prescribed value in place of the cycle light at 0845 hr. Only one test at any given level of illumination on a particular date.

levels of illumination the schooling activity rose continuously, though the fish continued to fluctuate in and out of the formation. The sequence of changes in the size of schools represented the combination of several activities: (1) fish withdrawing from a group to swim alone; (2) fish withdrawing from one group to join a passing group; or (3) solitary fish or subgroups coalescing into a larger group. As the histograms show, the schools frequently tended to go through a cycle of gradual enlargement followed by gradual reduction. While two schools might swim independently for several seconds and then dissolve, generally the two upon encounter would coalesce into a larger school. On the other hand, either schools or solitary fish might pass at near contact and show no signs of recognition.

The results of Series B (Fig. 4) were fundamentally the same as those of Series A insofar as schooling activity rose continuously with illumination. The

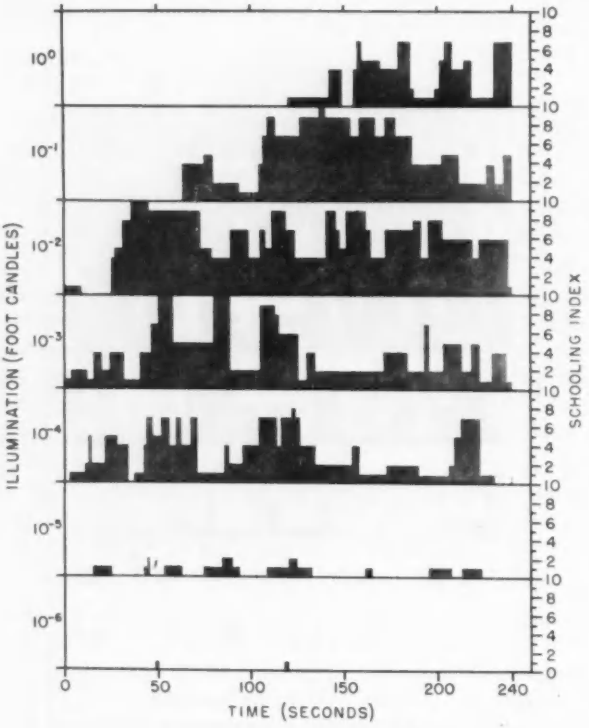

FIG. 4. *Series B.* Schooling responses of dark-adapted *Astyanax* at 2030 hr (2 hr in darkness) to sudden illumination by green light at a randomly prescribed value. Only one test at any given level of illumination on a particular date. Note the latent period before schooling which appears at 10^{-2} ft-c and becomes progressively longer at higher levels of illumination.

notable differences in Series B were: (1) the greater schooling activity at 10^{-5}, 10^{-4}, and 10^{-2} ft-c, and the contrary lower activity at 10^{-3} ft-c; (2) the increasing lapse of time between the moment of turning on the light and the formation of a school from 10^{-2} ft-c upwards; (3) the low schooling activity at 10^{-1} and 10^{0} ft-c.

The responses of dark-adapted fish at night to 10^{0} ft-c green light recorded over 8-min intervals on three different dates are compared in Fig. 5. The schooling index rose to a maximum only once for a few seconds during the first test. The schooling rate declined with each successive test, and the time lapse to the initial schooling increased. In contrast, when the fish were exposed to the brighter tungsten light ($10^{0.69}$ ft-c) at night, their response was generally consistent. They remained scattered and swam independently for about 4 min and then began to converge into a school. A durable school of six fish formed in 4–6 min from the time the light was turned on.

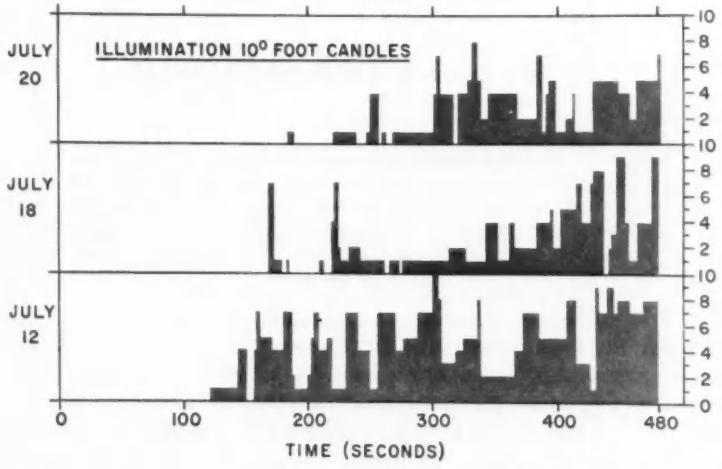

FIG. 5. *Series B.* Responses of dark-adapted *Astyanax* to sudden illumination by green light at 10^{0} ft-c on three different dates over an 8-min period. The latent period before schooling increased and the schooling rate decreased with repetition of the test.

When the light-adapted fish (Series C, Fig. 6) were exposed to the tungsten light at $10^{0.69}$ ft-c after 1 min in darkness they quickly formed a compact school of six, but after 150 sec, relaxed, scattered, and staged intermittent feinting attacks upon one another. When exposed to the green panels at 10^{0} ft-c, a lower illumination than the tungsten, the schooling activity was greater, in fact, the greatest obtained in any 4-min period. With further reductions of illumination the schooling decreased rapidly. The schooling at 10^{-4} ft-c was zero. However, considering the low schooling response at 10^{-3} ft-c, the true zero value for schooling would probably be near 10^{-3} ft-c.

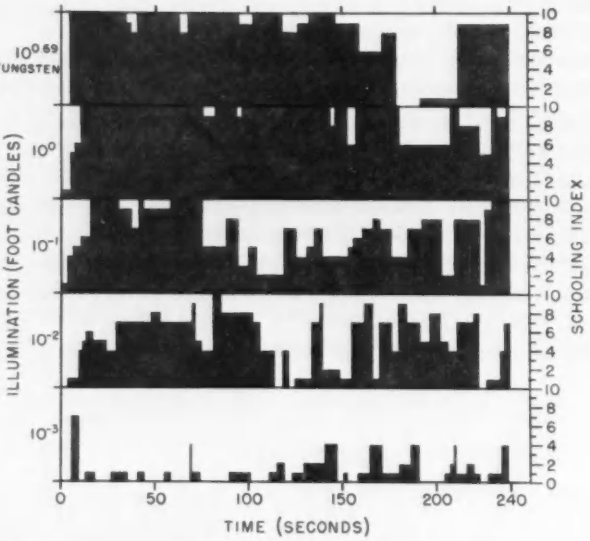

FIG. 6. *Series C.* Schooling responses of light-adapted *Astyanax* under sudden illumination during the day. The observations were made at decreasing levels of illumination. Prior to each test the tungsten light ($10^{0.69}$ ft-c) was turned off for 1 min and was again turned on for 1 min at the end of each test. The schooling response at 10^{-4} ft-c was zero.

Fish adapted to tungsten light, then exposed to 10^{-1} ft-c green light rather than the customary darkness at the instant the tungsten cycle light was turned off (Series D), formed a very compact school which did not begin to relax until about 4 min had elapsed (Fig. 7). The schooling response was greater than it had been in any other series at 10^{-1} ft-c, and was equal to or exceeded the values obtained at 10^{0} ft-c. By the end of the 15-min period the fish were calm and the schooling rate had fallen to the lowest value obtained in any series at an illumination of 10^{-1} ft-c, and was approximately equal to the value obtained under tungsten light at the beginning of the series. With further reductions in illumination, the schooling immediately declined and, excluding an apparently insignificant rise at 10^{-2} ft-c, continued to decline throughout each 15-min period. The series ended after the first 4-min observation at 10^{-5} ft-c.

Certain patterns were common in all tests. The schooling activity changed with each change of illumination. It is significant to note that the increases in schooling activity were represented not only by the greater durability of schools, but also by progressively larger schools at higher levels of illumination. It is also significant that the larger schools occurred infrequently at the lower levels of illumination.

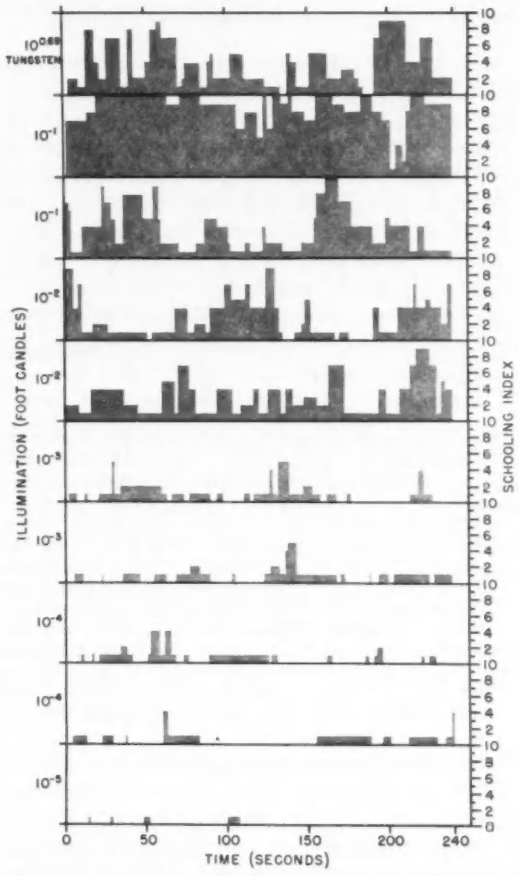

FIG. 7. *Series D.* Schooling responses of *Astyanax* at nightfall. The 4-min record under tungsten light was made 10 min before the cycle light went off. At the instant the cycle light went off, the green light was turned on at 10^{-1} ft-c and kept at that level for 15 min. The illumination was then suddenly reduced by 1 log unit and maintained at the new value for 15 min, and so on through the series. A record of the schooling activities was made during the first and last 4 min of each 15-min period excepting the single observation over the first 4 min at 10^{-6} ft-c.

The schooling rates from the four series are compared in Fig. 8.

The golden shiner, *Notemigonus*, behaved quite differently from *Astyanax* in some respects. In darkness, the fish were scattered throughout the aquarium swimming forward at a rate of 1–2 inches/sec and periodically remaining in one place for several seconds. At night, the dark-adapted fish, exposed to green

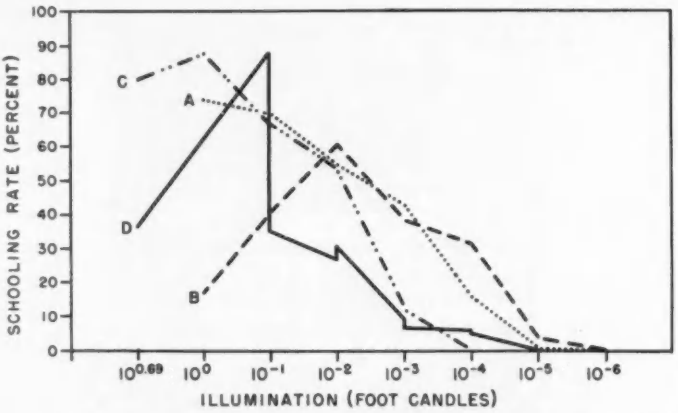

FIG. 8. Comparison of schooling of *Astyanax* under four procedures — Series A, B, C, D. Schooling rate equals the sum of the products of schooling index and time for each level of illumination, expressed as a per cent of the maximum which would be 10 × 240. All levels of illumination by green light, except $10^{0.69}$ ft-c by tungsten. Series A represents one test each day of dark-adapted fish to sudden illumination by green light at randomly prescribed values. Series B represents the same testing procedure as Series A excepting that the tests were performed at night on dark-adapted fish. Series C represents the responses of light-adapted fish to sudden reductions of illumination during the day. Series D represents the responses of fish to sudden reductions of illumination beginning at the end of the artificial day. In Series A, B, and C, each point represents a 4-min observation. In Series D, illumination remained at each level for 15 min, excepting the tungsten, with a 4-min observation at the beginning and end of each period except at 10^{-6} ft-c.

light at 10^{0} ft-c, stopped swimming abruptly and remained fixed in position for about 40 sec. Then the seven fish formed a compact school which remained almost motionless for 5 min. At the end of 6 min this compact school began to swim around the aquarium at a rate of about 4 inches/sec. Light-adapted fish in a compact school subjected to sudden darkness also stopped swimming abruptly and remained motionless as a school for 90 sec. Then they began to swim out of the school independently but the last fish remained in place for 300 sec. Thereafter they swam slowly and independently around the aquarium.

The schooling rates at low levels of illumination were substantially greater than those of *Astyanax*. At 10^{-5} ft-c, the schooling rate was 58% and at 10^{-6} ft-c, it was about 5%. These higher values probably derived from the slower swimming of the golden shiner. In fact, the fish often sat as a group in one place for several seconds. For example, in response to sudden illumination at 10^{-6} ft-c, four fish converged within 10 sec into a compact group and remained in place for 15 sec. At 10^{-5} ft-c, the schools swam very slowly, stopped intermittently, and frequently swam in a circle in one place. This behavior persisted for 4 min 10 sec. Then two of seven fish began swimming rapidly around the periphery of the aquarium. Note that in referring to a stationary group as a school, the terminology is not consistent with that of Breder (1959).

DISCUSSION

It is common knowledge that the schooling behavior of fishes in an aquarium is likely to change with time. Initially the fish swim in a compact school, but after a period of adjustment, they tend to scatter throughout the aquarium. Such behavior is an uncontested demonstration of the fact that illumination alone does not govern schooling behavior. Because compact schools are visually co-ordinated, illumination is a prerequisite condition for their occurrence, but the determining factors are the fright stimuli or stress conditions which have been discussed by Breder (1959) and others. Keenleyside (1955) systematically studied the relation between schooling and visual stimuli. Loukashkin and Grant (1959) increased the schooling of *Sardinops caerulea* by placing a predatory fish in the aquarium. This was probably a visual response but olfaction might also have been an instrumental factor. From the general statement that the intensity of schooling declines in the laboratory, it follows that nature abounds in fright stimuli and that the laboratory is a monotonous environment for the fish.

When fish are transferred from nature to the laboratory the dynamic characteristics of the environment which act as fright stimuli, such as the other animals and certain physical properties, are left behind. The action of the dynamic characteristics on the fish is essentially independent of the behavior of the fish. The transfer to the laboratory leaves behind also the static characteristics of the environment. Most important among these are the visibility in water and the extended dimensions of the habitat. The freedom for dispersal in the natural habitat provides a ready opportunity for visual isolation. In this case the action is dependent on the behavior of the fish. Typically the freedom for dispersal in nature exceeds the visibility. In the aquarium this is reversed; the freedom to disperse is less than the visibility. This reversal of relative dimensions may be fundamental to a proper explanation of the behavior of the fish in the aquarium. There is both physiological (Shlaifer, 1939) and behavioral (Steven, 1959) evidence that isolated fish are under stress. Visual isolation is a fright situation that one would expect fish to avoid, but they are not confronted with this problem in the confines of an aquarium. The significant biological question is whether fish in an extended environment with the monotony of the aquarium would disperse indefinitely or only to the limits of visibility. Is the fright of visual isolation an indelible attitude or would it, like the other fright responses, deteriorate with time? If it is indelible, one must conclude that any distribution in an aquarium under adequate illumination represents an integrated unit, a social group. Insofar as this unit would be a function of visibility, it would be pertinent to studies such as these which relate behavior to illumination. Without access to an aquarium of such extended dimensions this difficulty of defining an integrated unit cannot be overcome and one must arbitrarily focus interest on only one social formation, the compact school.

The values of minimum illumination for schooling for different species, summarized from the literature by Shaw (1961), seem unusually high, though one must recognize that schooling behavior is not characteristic of all fishes and varies in intensity among those species where it does occur. However, an evaluation

of these differences between species must be postponed until experimental procedures have been standardized. The changes in behavior with adaptation of fishes in general and the varying results on *Astyanax* show that there is no simple one-to-one correspondence between levels of illumination and intensity of schooling responses. Consequently, any statement on the minimum or threshold value of illumination for schooling is likely to be ambiguous.

It might be profitable for future studies to propose a theoretical schooling threshold as a guide in experimental designs. Because schooling depends on vision, one might assert that the threshold illumination for schooling is the same as the visual threshold. The fish must co-ordinate themselves with visual images of other fish. The initial assumption for experiments on any species would be that schooling ceases when the illumination falls below the visual threshold. If it ceases above the visual threshold, there is little profit in speculating on the possible roles of non-visual senses in maintaining aggregations in darkness. In short, the experimenter must establish the threshold value of illumination for schooling by showing that no combination of circumstances would induce schooling when illumination fell below that value. There is, of course, the companion problem of determining the visual threshold, but in any event this must be done before an explanation of schooling behavior can be finalized. The appropriate experimental techniques for determining threshold values of illumination for schooling may vary with different species. To date the responses of *Astyanax* have been gauged in relation to the single variable of changing illumination at different times in the diel cycle. I know that the schooling in response to a change in illumination can be increased by the simultaneous application of noise, but a critical evaluation of the role of noise or other joint stimuli remains to be done. One might also expect the response to vary with the number of fish in the experiment. It is often found that small schools show a preference for joining the larger of alternate schools. At high levels of illumination, larger groups could be analyzed from cinematographic records. At lower levels, functional but impractical technological aids must be by-passed for the human observer and the human observer has a very limited range of ability. The confusion in the aquarium rapidly becomes insurmountable when more than six fish are involved.

The differences in schooling thresholds reported in the literature are undoubtedly, in part, photometric errors derived from the general failure to relate the illumination within the water to that above the surface. However, the more significant part of the differences may stem from arbitrary definitions of schooling. For example, Ali (1959) defined minimal schooling for *Oncorhynchus* when more than two fish schooled persistently and maximal schooling when the largest school remained intact for more than 5 min. For species which behave like *Astyanax*, any criterion of schooling founded on arbitrary restrictions of the size and durability of the school would lead to confusion because the compact school at any adequate level of illumination may be a dynamic system fluctuating irregularly in size as members withdraw and return to the group. There may be no 100% participation in a compact school over any prolonged period of time

under laboratory conditions though I think it could be obtained over at least a 4-min period with different procedures on *Astyanax*. If percentage composition with temporal restrictions is taken as the criterion for schooling, then maximal schooling, that is, total group participation, may have to be equated with some value less than 100% depending on the species.

In using small numbers of fish in laboratory experiments it is quite probable that the schooling rates would differ significantly for two groups owing to idiosyncracies of certain individual fish. Ignoring time and allowing for idiosyncracies, perhaps a school of four *Astyanax* in this particular group represented maximum schooling. In this event, one could define the threshold illumination for maximum schooling at 10^{-4} ft-c. Again ignoring time, but specifying 100% participation in the school, the threshold value was 10^{-3} ft-c. With the temporal restriction of 4 min, the threshold value rose to 10^{-1} ft-c. It seems to me that the least subjective and therefore most useful measure of schooling can be obtained only by recording the total behavior for some time from the moment of changing the stimulus situation. To further clarify the results one might distinguish between the illumination threshold for schooling (maximum school) and the illumination for threshold schooling (minimum school).

With the results limited to only one set of observations under each series it would be premature to evaluate the testing procedures beyond noting that the maximal schooling responses at lower levels of illumination were obtained on dark-adapted fish and at the higher levels of illumination on light-adapted fish. Certain peculiarities between the responses of dark-adapted fish at night and during the day can be correlated with the behavioral and retinomotor cycles that persist in continuous darkness (John and Haut, 1964). We still do not know whether it was a visual adjustment alone or some more complex behavioral adjustment that determined the latent period before schooling in nocturnally dark-adapted fish. The retina in darkness at night was dark-adapted but dur ng the day it was in an intermediate condition and most nearly approximating the light-adapted state. The undelayed schooling responses of dark-adapted fish during the day (Fig. 3) being a measure of visual ability, showed that both rods and cones were exposed to the incident light. The retina was in the unique condition of receiving and transmitting adequate visual information to the central nervous system at any level of illumination.

The schooling responses of light-adapted fish with sudden reductions of illumination (Fig. 6) established the cone threshold in the range of 10^{-3} ft-c. The rod threshold, established from the other tests, was in the range of 10^{-6} ft-c. Since these values were determined by behavioral end-points, they must be considered tentative. The absolute values may be somewhat lower. The responses of dark-adapted fish at night suggest that the upper limit of illumination in which rods continue to function is in the order of 10^{-2} ft-c. This was the level at which a latent period before schooling was first detected. Therefore, the mesopic range for this species is roughly from 10^{-3} to 10^{-2} ft-c.

There are other peculiarities in the responses which cannot be explained without further work. Among these was the relatively weak schooling of fish

at night under 10^0 ft-c green light (Fig. 5). It was not that the light was too bright. They schooled well under this light at other times in the diel cycle. Moreover, they schooled well at night under tungsten light at $10^{0.69}$ ft-c. The failure of the fish to form a maximal school under 10^0 ft-c green light at night may mean that they were excessively frightened and were seeking to escape the aquarium. An interesting threshold is implicit in this statement, namely, the threshold at which fright ceases to induce schooling and releases the escape behavior. There may be some problems in terminology. Keenleyside (1955) designated fleeing motivation as the cause for both schooling and escape behavior.

The results from the collective tests point up several things for studies on schooling. First, if fish naturally do not school at night, the schooling should cease in the laboratory at a level of illumination well above the visual threshold if the fish are calm and if the light is gradually reduced. Thus schooling would cease at a level of illumination higher than that at which it can be made to occur. Secondly, since the maximal schooling may occur in the short interval following the change in stimulus and decline thereafter, the investigator must begin observations immediately after the stimulus is altered if a record of maximal schooling is desired. Thirdly, either the technique of flash photography or that of suddenly switching on the room lights to determine what fish are doing in darkness or low-level illumination can provide a range of possible conclusions depending on the coincidence of behavior of the fish and the flash of light. The greatest probability, however, at low levels of illumination is that the instantaneous record would show no schooling. Finally, whatever the means one employs to induce schooling, the duration of the experiments may have to be restricted to obtain maximal results. Keenleyside (1955) found that fish induced to school by visual stimuli quickly accommodated to the new condition, as *Astyanax* did to a change in illumination.

The gradual decline in the schooling of *Astyanax* correlated with reductions in illumination is an interesting relationship and may be a general pattern among fishes. Steven (1955) described it for *Hepsitia stipes* and *Bathystoma rimator*. In attempting to explain it, perhaps the most pertinent thing to repeat is that *Astyanax* does not naturally school at night. It may be remarkable that any schooling at all was obtained. At best, the performance of the fish in the laboratory indicates what they are capable of doing in nature. Schooling would signify that they saw one another. In spite of mutual visibility among them, in an aquarium furnished with plants and other objects, the fish might settle to the substrate at night as they do in nature. On the other hand, the performance of *Notemigonus* suggests that the failure to obtain large durable schools of *Astyanax* in dim light was the result of the combination of a temperamental species and an abnormal situation. Aside from these restrictions, since schooling depends on vision, its decline with decreasing illumination could probably be explained by the accompanying reduction of visual acuity and visibility, the limits of which vary in accordance with a third factor, the swimming speed of the fish. The implication here is that each fish in a large school must have adequate visual conditions to co-ordinate its position with more than one other fish. How

many other fish it might require for adequate co-ordination is not known. In decreasing light it could maintain a durable co-ordination only with diminishing numbers of fish. Under bright light a fish could depart from the school to a distance limited by the aquarium but it would never exceed the visual range, and it could readily return to the school. As the illumination decreased, the visibility and visual acuity would decrease and the opportunity for visual isolation would increase. Once isolated, a fish could not again enter the school until it happened to swim within visual range of it. In an extended environment the chance of an isolated fish encountering another fish would be low. At the other extreme, a sufficiently small aquarium would prohibit dispersal. Between these extremes the frequency of recombinations of subgroups or isolated fish would be determined by the dimensions of the aquarium to the extent that chance determines the encounters.

The importance of the swimming speed of the fish in determining schooling rates was demonstrated by the greater frequency and durability of schools among slower swimming *Astyanax* and was especially well exhibited by *Notemigonus*. As is true of *Astyanax*, littoral species may avoid voluntary dispersal at night by retiring at sunset to some particular territory. Pelagic groups could be maintained if they compensated for the lowering of visual performance with decreasing light by reducing their locomotory activity.

To answer the questions on the causes of alternations in the size of schools one would need to know why fish depart from a compact school. The fact that groups of fishes in nature are predominantly homotypic signifies that the individuals have some fundamental quality of attractiveness regardless of the mechanisms that bring them together. However, it is apparent that within a group the attractiveness must be countered by some opposing quality as postulated by Parr (1927). The resultant balance between the two qualities, attraction and repulsion, determines the distance between fish. This distance appears to be species-specific and may result in one of the stable group configurations defined by Breder (1959). Insofar as the reference point of attraction and repulsion for one fish is another conspecific individual, which is the stimulus, these words can be substituted essentially for the approaching and withdrawing reactions of Schneirla (1959). The only necessary assumption to complete this analysis is that the intensity of the stimulus (conspecific fish) is a function of the distance between fish. At low intensity it is attractive and at high intensity it is repulsive. The final distance would be a measure of the stability characteristic. Any deviation away from this would represent instability and a transient configuration. The conditions for stability cannot be generalized easily. In the laboratory, under monotonous conditions, *Astyanax* assumes the stable distribution of a loose aggregation.

To define instability appropriate for *Astyanax*, one might assume that the stimulus, like a magnetic field, decreases in intensity as the distance between fish decreases, and is always repulsive, never becoming attractive. Then the approaching and withdrawing reactions are governed by two repulsive forces acting on each

fish. One is centered in the conspecific fish, the other in the surrounding environment.

Under monotonous environmental conditions, the distance between fish becomes maximal and is determined by the visibility. The cardinal assumption is that the fish avoid visual isolation from conspecific individuals. As a change in conditions increases the environmental repulsion, the fish are forced to approach one another but with a consequent increase in the repulsive force between fish. This is an unstable balance. Compact schools of *Astyanax* represent the maximal approaching reaction, but this is a passive consequence of active retreat from a repulsive environment. If the conditions remain constant, the school enters the dynamic stage with individuals repeatedly departing and returning. Apparently this dynamic behavior represents gradual accommodation to the conditions. Complete accommodation is indicated when the fish are again scattered and relatively quiet, which is to say, they have returned to the stable social configuration. The fighting among fish after accommodation in a small aquarium but not in a large aquarium is an illustration of these reactions. It is the result of unstable conditions where the repulsion between fish remains at a high level and there is insufficient space to permit dispersal to the stable interval. I think *Astyanax* exhibits these reactions notably because it is constitutionally pugnacious and only conditionally forms a compact school.

In conclusion of her study, Shaw (1961) stated that we must know how much light enters the eye of the fish before we can satisfactorily give meaning to the question of the minimum light for schooling. The amount of light entering the eye does not determine schooling activity because schooling depends on mutual visual recognition between fish, and a visualized object is not defined in absolute terms of brightness but in relative terms. A white fish against a white background would be less visible than a black fish against a white background regardless of the level of illumination and the amount of light entering the eye.

The source of illumination is the light striking the surface of the water. If we did nothing more than relate schooling behavior to incident radiation we would at least establish a useful first approximation. To express the schooling responses directly in terms of available illumination, we must take measurements within the ambient medium because the distribution of light below the surface of the water is determined by many factors, the more important being: suspended materials, depth of water, and color of the bottom. To be of absolute value, the surface illumination must be related to the conditions for vision below the surface. Since vision depends on relative illumination this would not be a simple problem. In nature, except over a light-colored bottom, one would not expect the downward illumination to be greater below than above the surface of the water. This situation obtains in a white aquarium. It may occur in other aquaria depending upon the amount of internal reflection. If the apparatus could be designed so that the internal illumination was always less than the external illumination, measurements of the illumination incident upon the surface of the water would at least be conservative estimates of the limitations of visual processes.

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REFERENCES

ALI, M. A. 1959. The ocular structure, retinomotor and photobehavioral responses of juvenile Pacific salmon. *Canadian J. Zool.*, **37**: 965–996.

ATZ, J. W. 1953. Orientation in schooling fishes. *In* Proceedings of a Conference on Orientation in Animals, Feb. 6, 7, 1953, Washington. Washington, D.C., Office of Naval Research Department of the Navy.

BREDER, C. M., JR. 1959. Studies on social groupings in fishes. *Bull. Amer. Mus. Nat. Hist.*, **117**: 393–482.

ENGSTROM, R. W. 1960. Absolute spectral response characteristics of photosensitive devices. *RCA Review*, **21**: 184–190.

JOHN, K. R., AND M. HAUT. 1964. Retinomotor cycles and correlated behavior in the teleost *Astyanax mexicanus* (Fillipi). *J. Fish. Res. Bd. Canada*, **21**: 591–595.

KEENLEYSIDE, M. H. A. 1955. Some aspects of the schooling behaviour of fish. *Behaviour*, **8**: 183–248.

LOUKASHKIN, A. S., AND N. GRANT. 1959. Behaviour and reactions of the Pacific sardine, *Sardinops caerulea* (Girard), under the influence of white and colored light and darkness. *Proc. Calif. Acad. Sci.*, **29**: 509–548.

MOULTON, J. M. 1960. Swimming sounds and the schooling of fishes. *Biol. Bull.*, **119**: 210–223.

PARR, A. E. 1927. A contribution to the theoretical analysis of the schooling behaviour of fishes. *Occasional Papers Bingham Oceanogr. Coll.*, No. 1, pp. 1–32.

SCHNEIRLA, T. C. 1959. An evolutionary and developmental theory of biphasic processes underlying approach and withdrawal. *In* Nebraska Symposium on Motivation. University of Nebraska Press.

SHAW, E. 1961. Minimal light intensity and the dispersal of schooling fish. *Bull. Inst. Oceanogr.*, No. 1213, 8 pp.

SHLAIFER, A. 1939. An analysis of the effect of numbers upon the oxygen consumption of *Carassius auratus*. *Physiol. Zool.*, **12**: 381–392.

STEVEN, D. M. 1959. Studies on the shoaling behaviour of fish. I. Responses of two species to changes of illumination and to olfactory stimuli. *J. Experiment. Biol.*, **36**: 261–280.

Dissolved Calcium Carbonate in the Labrador Sea[1]

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ABSTRACT

Calcium and carbonate ion concentrations have been measured in the upper 100 m in an area southwest of Greenland. The values indicated that these waters were about 100% supersaturated with respect to calcite and about saturated with respect to aragonite. Because of the large influx of CO_2 from the air in this region it was concluded that considerable CO_2 must have been utilized or there would have been evidence for the transport of this gas to lower levels.

INTRODUCTION

The extent of saturation of sea water with calcium carbonate is still largely unknown, in part because of the uncertainty of its solubility product but also because large-scale measurements of both $[Ca^{++}]$ and $[CO_3^{=}]$ in the oceans are still lacking. Whether or not saturation exists is of importance from the point of view of sedimentation and is also an important facet of the carbonate equilibria in sea water and the photosynthetic processes of marine organisms.

Calcium carbonate should precipitate as calcite from an aqueous solution in which the product of the calcium and carbonate-ion concentrations $[Ca^{++}][CO_3^{=}]$ exceeds the solubility product for calcite, as described by Sverdrup *et al.* (1942). Cloud (1962) has shown that the more soluble aragonite can precipitate if the product $[Ca^{++}][CO_3^{=}]$ rises to very high values such as exist on the Great Bahama Banks. Since sea water contains carbonic acid and its dissociation products, the following equilibria must be satisfied:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad K_h$$

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad K_1$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{=} \qquad K_2$$

Obviously $[CO_3^{=}]$ is not a conservative quantity but can be altered by changes in pH, CO_2 partial pressure, temperature, pressure, and salinity. While it is not possible at present to measure $[CO_3^{=}]$ directly in sea water, the quantity $[HCO_3^-] + 2[CO_3^{=}] + [H_2BO_3^-]$, termed the alkalinity, can be measured by titration

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with a strong acid such as HCl, as described by Wattenberg (1933). By subtracting the small contribution made by the borate ion, the expression for the carbonate alkalinity, A, becomes:

$$A = [HCO_3^-] + 2[CO_3^=]$$

which, combined with the expression for the second dissociation of carbonic acid

$$\frac{[H^+]\,[CO_3^=]}{[HCO_3^-]} = K_2$$

becomes:

$$[CO_3^=] = \frac{A\,K_2}{[H^+] + 2K_2}$$

where: A is the carbonate alkalinity obtained by titration of sea water and subtraction of the contribution from $H_2BO_3^-$.

K_2 is the second dissociation constant of carbonic acid as determined by Lyman (1957). This is a function of temperature, salinity, and depth.

$[H^+]$ is derived from the *in situ* pH. A knowledge of $[Ca^{++}]$ obtained from analysis then allows the calculation of $[Ca^{++}]\,[CO_3^=]$.

EXPERIMENTAL

Between May 21 and June 17, 1963, CNAV *Sackville* participated in a cruise in the area southwest of Greenland in connection with a program initiated by the International Commission for the Northwest Atlantic Fisheries. On this cruise, pH, alkalinity, temperature, and salinity data were collected which allowed the calculation of $[CO_3^=]$ for about 160 samples from the upper 100 m. These samples were later analyzed for $[Ca^{++}]$ ashore. The results presented here represent a general picture of the calcium carbonate system in the surface waters studied.

Water samples were taken in Knudsen type bottles fitted with reversing thermometers and internally coated with "Teflon".

Chlorinities were measured on an NIO conductivity salinometer.

The pH measurements were made on a Radiometer pH Meter Model 4, using a glass electrode which had been standardized with the following buffers:

a. 0.05*M* potassium hydrogen phthalate
   pH = 4.00 at 20–25°C

b. 0.025*M* KH_2PO_4 + 0.025*M* Na_2HPO_4
   pH = 6.87 at 20–25°C

c. 0.05*M* $Na_2B_4O_7$
   pH = 9.18 at 25°C.

Measurements of pH made at room temperature were converted to *in situ* pH values by means of tables from Strickland and Parsons (1960).

Samples for alkalinity measurements were filtered and 12.50 ml of 0.0100*N* HCl was added to 50.00 ml of sample. The final pH was measured and converted to carbonate alkalinity by means of tables supplied by Dr K. Grasshoff of Kiel, West Germany, and conversions to P_{CO_2} were made by means of tables from Strickland and Parsons (1960).

The calcium content was determined by titration of 25-ml samples with 0.150*M* ethylenediaminetetraacetic acid (EDTA) at pH 12 (with NaOH) using Calver II[2] as an indicator. The EDTA was standardized with synthetic sea water containing known concentrations of Ca^{++} and Sr^{++} (Sr^{++} also reacts with EDTA).

RESULTS

The stations occupied are shown in Fig. 1. There was considerable "patchiness" in the values of $[Ca^{++}]$ $[CO_3^{=}]$ and no horizontal contouring was attempted. The average values for the 23 stations for $[Ca^{++}]$, $[Ca^{++}]/Cl‰$, P_{CO_2}, $[Ca^{++}]$ $[CO_3^{=}]$, and per cent saturation of $CaCO_3$ are shown in Table I for the standard depths sampled.

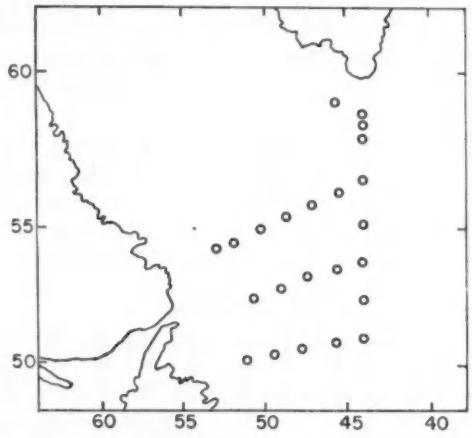

FIG. 1. Station positions occupied in the Labrador Sea.

The 100% saturation figures were obtained from the solubility data of Wattenberg and Timmerman (1936). These workers equilibrated sea water with calcite and measured the $[Ca^{++}]$ $[CO_3^{=}]$ ion product in the resulting solutions. Their values for sea water of 19‰ chlorinity varied from 0.81×10^{-6} at 0°C to 0.74×10^{-6} at 10°C. Revelle and Fleming (1934) obtained much larger values for the solubility product of K_{sp}, but their results were shown by Wattenberg and Timmerman to have been obtained in solutions in which equilibrium with calcium carbonate had not been attained.

[2]Obtained from Hach Company, Ames, Iowa, USA.

TABLE I. Average of results for 23 stations at each of seven depths.

Depth (*m*)	$[Ca^{++}]$ ($\times 10^2$ M)	$[Ca^{++}/Cl]_{\text{‰}}$ (*g/g*)	$[CO_3^{=}]$ ($\times 10^4$ M)	P_{CO_2} ($\times 10^4$ atm)	$[Ca^{++}][CO_3^{=}]$ ($\times 10^8$ M^2)	Per cent saturation of $CaCO_3$
0	1.04	0.0217	1.40	3.2	1.45	190
	±0.02	±0.0005	±0.05	±0.2	±0.07	±10
10	1.04	0.0217	1.40	3.2	1.45	190
20	1.04	0.0217	1.45	3.2	1.50	190
30	1.04	0.0217	1.50	3.0	1.55	200
50	1.05	0.0218	1.35	3.3	1.40	180
75	1.05	0.0218	1.35	3.4	1.40	180
100	1.05	0.0218	1.30	3.4	1.35	180

DISCUSSION

The uniform values of the variables shown in Table I indicate good vertical mixing of all components determined in the upper 100 m. The $[Ca^{++}]\,[CO_3^{=}]$ ion product is about twice as great as the solubility product for calcite and about equal to that for aragonite reported by Cloud (1962). One therefore would expect calcite to be precipitated in such an area although Wattenberg has shown that such precipitation may be very slow to occur. The factors governing precipitation are shown in Appendix I.

Since this is an area which, according to Dingle (1954) has a net invasion rate of CO_2 from the air of about 5×10^{-3} mole/m²-sec, an efficient process must exist for removing this CO_2 from the upper 100 m of water. Transfer of CO_2 to lower depths would lead to a gradient of P_{CO_2} with depth and since this is not evident in Table I, the CO_2 must be utilized in the upper 100 m. If photosynthesis alone is removing the CO_2 absorbed from the air it must account for the production of about 0.15 g/sec of carbohydrate under each square metre water column.

Any process which removes calcium carbonate from the water would add to the production of CO_2 by shifting the equilibrium below to the right:

$$2HCO_3^- \rightleftharpoons H_2O + CO_2 + CO_3^=$$

This would imply an even greater photosynthesis activity than the above figures indicate if $CaCO_3$ is either precipitated inorganically or abstracted by calcareous plankton.

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REFERENCES

CLOUD, P. E., JR. 1962. Behaviour of calcium carbonate in sea water. *Geochim. Cosmochim. Acta.*, **26**: 867–884.

DINGLE, A. N. 1954. The carbon dioxide exchange between the North Atlantic Ocean and the atmosphere. *Tellus*, **6**: 342–350.

LYMAN, J. 1957. Buffer mechanism of sea water. Ph.D. Thesis, University of California, Los Angeles, California. 196 pp.

REVELLE, R., AND R. H. FLEMING. 1934. The solubility product constant of calcium carbonate in sea water. *Proc. 5th Pacific Sci. Congr.*, 1933, **3**: 2089–2092.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1960. A manual of sea water analysis. *Bull. Fish. Res. Bd. Canada*, No. 125, 185 pp.

SVERDRUP, H. U., M. W. JOHNSON AND R. H. FLEMING. 1942. The oceans, their physics, chemistry, and general biology. Prentice Hall Inc., New York. 1087 pp.

WATTENBERG, H. 1933. Über die titrationsalkalinität und den Kalziumcarbonatgehalt des Meerwassers. *Wiss. Ergebn. Deut. Atlant. Exped. 'Meteor'* 1925–27, **8**(2):122–131.

WATTENBERG, H., AND E. TIMMERMAN. 1936. Über die Sättigung des Meerwassers an $CaCO_3$, und die anorganorgene Bildung von Kalksedimenten. *Ann. Hydrogr. Mar. Meteor.*, pp. 23–31.

APPENDIX

FACTORS GOVERNING PRECIPITATION OF CALCIUM CARBONATE

Two crystalline forms of calcium carbonate may be precipitated from sea water. The less soluble form, calcite, is stable at the normal temperatures and pressures occurring in the sea and will precipitate (often only very slowly) from sea water for which the ion product of $[Ca^{++}]\,[CO_3^{=}]$ exceeds the solubility product of calcite, which is a constant at any given temperature, pressure, and salinity. The more soluble form, aragonite, is metastable and will precipitate from sea water for which the ion product of $[Ca^{++}]\,[CO_3^{=}]$ exceeds the solubility product of aragonite. A third, highly unstable form, vaterite, is known but is not important for our discussion. Since the free energy change ΔF° for the conversion of aragonite to calcite is ~ -300 cal/mole, a substitution into the well known expression for the equilibrium constant K gives:

$$\Delta F^\circ = \sim -300 = -RT\,ln\,K = -RT\,ln\,\frac{K_{sp}\text{ (calcite)}}{K_{sp}\text{ (aragonite)}}$$

from which we find that the solubility product of aragonite is about 1.6 times that for calcite.

A clear distinction must be made between an ion product which in this case is the product of the analytical concentrations of $[Ca^{++}]$ and $[CO_3^{=}]$ and a solubility product which is a constant at any given temperature, pressure, and salinity. The ion product may be greater than, equal to, or less than the solubility product depending on whether the solution is supersaturated, saturated, or under-saturated with the component in question. In sea water, if the $[Ca^{++}]\,[CO_3^{=}]$ ion product is greater than the solubility product of aragonite, then aragonite will precipitate until the ion product equals the solubility product for aragonite; then calcite will precipitate until the $[Ca^{++}]\,[CO_3^{=}]$ product equals the solubility product of calcite. This sequence of reactions is predicted by the empirical rule of Ostwald. Any precipitated aragonite should then be converted to calcite by some other process.

It should be understood that the above discussion holds only for physicochemical precipitation. Organisms can precipitate calcite or aragonite against thermodynamic gradients but must consume energy to do so.

The Volatile Sulphur Compounds of Oysters[1]

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ABSTRACT

The compound responsible for the characteristic odour of fresh Pacific oysters, *Crassostrea gigas* (Thunberg), has been identified as dimethyl sulphide [$(CH_3)_2S$], by the formation of mercury salts, and by infrared analysis and gas chromatography. The effect of bacterial action on the oysters has been observed and a number of the volatile organo-sulphur decomposition compounds, produced during room storage at 20–21°C, have been identified by gas chromatography and by the formation of their lead and mercury salts.

INTRODUCTION

Yueh (1961) isolated dimethyl sulphide, hydrogen sulphide, methanethiol, ammonia, diacetyl and monocarbonyl compounds from boiled oysters and a compound from raw oysters which he considered to be dimethyl sulphide because it formed a mercuric chloride adduct which, on reaction with sodium hydroxide, gave an odour of dimethyl sulphide. However, as marine products undergo rapid decomposition upon removal from the sea and as the oysters involved in Yueh's work were obtained commercially about 1000 miles from the sea, it was considered that some of these compounds could have been due to decomposition. Thus the present study was carried out to determine the nature of and to positively identify the characteristic odour of freshly harvested Pacific oysters.

EXPERIMENTAL

Pacific oysters, *Crassostrea gigas* (Thunberg), were obtained from Mud Bay, Ladner, B.C., and Fanny Bay, Vancouver Island, B.C., during the winter months (October to May) when the organoleptic quality is considered to be excellent. Immediately after shucking, the oysters were washed and put into 1-gal cans which were then sealed and immersed in ice for transportation to the laboratory. The isolation of volatile compounds from the fresh oysters was usually commenced within 1 hr and not later than 4 hr after shucking.

Collection of Volatile Compounds from Oysters

Upon arrival at the laboratory the oysters were quickly homogenized while cold and then placed in a 22-litre round-bottomed flask. Two litres of water was added to 2 gal of oysters and nitrogen was bubbled through the continuously stirred mixture while it was allowed to warm up to room temperature (21°C).

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The effluent gases were led through a 40-cm water-cooled reflux condenser and then through a series of liquid-bubbling traps which contained reagents to precipitate specific classes of compounds (Challenger, 1959). The reagents used were: 2% lead acetate for hydrogen sulphide; 4% aqueous mercuric cyanide for thiols; 3% aqueous mercuric chloride for sulphides; and a 1% methanolic-hydrogen chloride solution of 2,4-dinitrophenylhydrazine for carbonyl compounds, methanol being added regularly to maintain the concentration. With the exception of a copper tube, which connected the gas inlet tube to the nitrogen cylinder, the apparatus and connections were made of glass.

Bacterial Study on Oysters

Viable bacterial counts were made at regular time intervals during the removal of volatiles and notes made of the oyster odour and the precipitates in the series of traps. Bacto nutrient agar–3% (W/V) sodium chloride was the medium used in the preparation of plates for aerobic bacterial counts. Anaerobic Agar (BBL)–3% (W/V) sodium chloride medium was used in the preparation of Brewer plates for anaerobic bacterial counts. Plates were incubated at 25°C for 72 hr. The lead acetate trap was omitted in this experiment.

Characterization of Mercuric Chloride Adduct

The precipitate formed in the mercuric chloride solution during the first 12 hr of collection was washed, dried, and examined as follows: A 2-ml glass test tube containing 1 mg of the precipitate was sealed with a rubber septum, evacuated, and then filled with dry nitrogen. With the aid of a syringe, 0.2 ml of 2*N* HCl was injected into the tube. The mixture was warmed to boiling and a 1.0-ml sample of vapour was withdrawn into a gas syringe for analysis by gas chromatography.

A sample for infrared analysis was obtained as follows: Ten milligrams of the mercuric chloride complex precipitated from oyster vapours was added to 0.5 ml of 2*N* HCl in a 10-ml test tube. The mixture was heated to 50°C and dry nitrogen was passed through the system at a rate of 1 ml/min. The vapours were collected by bubbling for 10 min through 5 μl of spectrograde dichloromethane in a glass tube, 50 mm long and 2 mm ID in a dry-ice bath. The tube was then cut to 20 mm and the contents transferred, first to a 20-gauge hypodermic needle attached to a micro-burette[3] and then via the hypodermic needle to an ultra-micro liquid infrared cell[4].

Collection of Volatile Compounds Produced Upon Storage of Oysters at 20–21°C

One-gallon samples of oysters were held at 20–21°C in a closed 6-litre flask fitted with a rubber septum and a manometer to enable the atmosphere of either air or nitrogen to be adjusted to atmospheric pressure. At 1-hr intervals 50 ml of headspace gas was removed with a syringe and transferred to the gas chromatographic system via a gas sampling valve assembly.

[3]Microchemical Specialities Co., Berkeley, California.
[4]Beckman Instruments Inc., Fullerton, California, Part No. 28714.

Air was passed at 20 ml/min over 1 gal of homogenized oysters, in a three-necked 6-litre flask. The mixture was stirred slowly and the exit gases passed through a trap immersed in liquid nitrogen. Periodically the trap was allowed to warm sufficiently to allow removal of the liquified oxygen. A similar experiment was conducted using nitrogen in place of air. The traps were removed and sealed with a rubber septum which allowed samples to be withdrawn with a syringe for examination by gas chromatography.

The infrared analysis was carried out on a Beckman IR-4 spectrophotometer equipped with a micro-beam condenser. A Barber-Colman model 10 gas chromatograph was used for the analysis of the volatile components. Three different columns were used, the stationary phases being 10% Carbowax 1500[5], 20% tritolyl phosphate and 30% tritolyl phosphate[6], each supported on 60–80-mesh Chromosorb W[7] which had been washed with acid and base and treated with dimethyldichlorosilane. The glass columns were each 180 cm in length and 4 mm ID. A Sr^{90} argon ionization cell was used for detection and operated at 1000 v at 146°C. The columns were held at 70°C and the flash heater at 125°C. The carrier gas flow was 50 ml/min.

RESULTS

The viable bacterial counts (Table I) indicated that the development of an undesirable odour in the oysters was correlated with an increase in the bacterial population and the production of hydrogen sulphide and probably of other thiols. However, because a control experiment was not conducted under sterile conditions, it cannot be concluded that the production of the undesirable odour was

TABLE I. Effect of holding oysters at 21°C under nitrogen on the bacterial count, odour, and production of sulphur compounds.

Time (*hr*)	Viable bacterial count (*bacteria* × 10^6/*gram*)		Contents of mercuric cyanide trap	Odour of oysters
	Aerobic	Anaerobic		
0	0.02	0.002	nil	typical
6	0.02	0.002	nil	typical
12	0.05	0.009	nil	typical
18	4.50	0.90	nil	slightly off
24	23.5	4.0	grey ppte.	slightly unpleasant
28	32.0	9.0	black ppte.	distinctly unpleasant

the consequence of microbiological action. During the collection of the volatile compounds from oysters, it was observed that a precipitate formed in the mercuric chloride solution immediately after the nitrogen flow commenced. This indicated the presence of a sulphide or sulphides. It was also observed that after approximately 18 hr when precipitates began to appear in the other traps the

[5]Polyethylene glycol, Carbide and Carbon Chemicals Co., New York, N.Y.
[6]May and Baker Ltd., Dagenham, England.
[7]Johns-Manville Co., Manville, New Jersey.

oysters began to develop an unpleasant odour which was not typical of the fresh product.

Identification of Dimethyl Sulphide $(CH_3)_2S$

The regenerated vapours from the mercuric chloride complex were gas chromatogrammed on two different columns, tritolyl phosphate and Carbowax 1500. The only peaks observed were those with retention times corresponding to hydrogen chloride and to dimethyl sulphide. The peak corresponding to dimethyl sulphide required full attenuation of the detection system, i.e. minimum sensitivity, showing that if any other sulphides were present their concentration would have been in the order of 1/5000th of that of dimethyl sulphide. When this compound was eluted from the gas chromatographic system, its odour was like that of fresh oysters and of dimethyl sulphide. When the eluting gas was bubbled into an aqueous solution of mercuric chloride a white precipitate was formed.

The identity of this compound was confirmed by infrared analysis. The infrared spectrum was identical to that obtained from an authentic sample of freshly distilled dimethyl sulphide. An identical spectrum was also obtained from the compound released when the mercuric chloride complex of authentic dimethyl sulphide was treated in exactly the same manner.

The volatile compounds produced when oysters were held at 20–21°C were examined by gas chromatography. For the first 2 or 3 hr only dimethyl sulphide was detected. Then thiols began to make their appearance. Figure 1 shows the

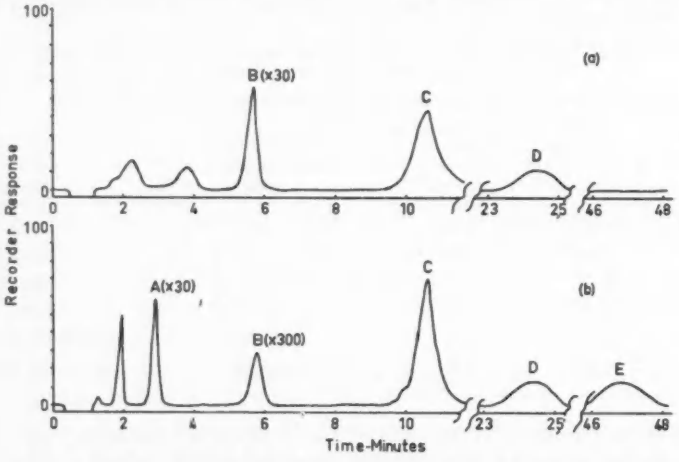

FIG. 1. Gas chromatograms of headspace vapours over oysters held at 20–21°C for 20 hr, (a) under nitrogen atmosphere, and (b) under air. A — Methanethiol; B — Dimethyl sulphide; C — 1-Propanethiol; D — 1-Butanethiol; E — Dimethyl disulphide.

Packing: 30% tritolyl phosphate on 60–80-mesh chromosorb "W". *Column:* glass. L = 180 cm. ID = 4 mm. *Temperature:* 70°C. *Detector:* Sr^{90} argon ionization cell, operating voltage = 1000 v, temperature = 146°C. *Flash heater:* 125°C. *Carrier gas:* Argon, flow rate 50 ml/min.

chromatograms run on the 30% tritolyl phosphate column that resulted from the final sample taken after 20 hr from air and nitrogen atmospheres. Similar chromatograms were obtained on the other columns and by comparison of the retention times with those of authentic compounds the following tentative identities were made: peak A, methanethiol; peak B, dimethyl sulphide; peak C, 1-propanethiol; peak D, 1-butanethiol; and peak E, dimethyl disulphide. The remaining peaks could not be identified as sulphur-containing compounds although a large number of reference compounds was tested. Of the columns used the Carbowax column gave good separation of a mixture of thiols or a mixture of alkyl sulphides but not a mixture of both. The 30% tritolyl phosphate column gave good resolution of a mixture of thiols and sulphides as illustrated by the retention times given in Table II.

TABLE II. Retention times of sulphides and thiols on a 30% tritolyl phosphate gas chromatographic column 180 cm in length and 4 mm ID at 70°C and argon flow rate of 50 ml/min.

Compound	Formula	Retention time (*min*)
Hydrogen Sulphide	H_2S	1.4
Methanethiol	CH_3SH	2.9
Ethanethiol	C_2H_5SH	4.8
Dimethyl sulphide	$(CH_3)_2S$	5.7
2-Propanethiol	$CH_3CHSHCH_3$	6.4
2-Methyl-2-propanethiol	$(CH_3)_3CSH$	7.3
1-Propanethiol	$CH_3(CH_2)_2SH$	10.6
Methyl ethyl sulphide	$CH_3SC_2H_5$	11.6
2-Butanethiol	$CH_3CHSHCH_2CH_3$	15.0
2-Methyl-1-propanethiol	$(CH_3)_2CHCH_2SH$	17.2
Diethyl sulphide	$(C_2H_5)_2S$	21.5
1-Butanethiol	$CH_3(CH_2)_3SH$	24.4
Methyl *n*-propyl sulphide	$CH_3SC_3H_7$	24.7
3-Methyl-1-butanethiol	$(CH_3)_2CH(CH_2)_2SH$	39.7
Dimethyl disulphide	CH_3SSCH_3	46.8
Methyl *n*-butyl sulphide	$CH_3SC_4H_9$	55.8

At the time of withdrawal of the final sample the two batches of oysters had distinctly different odours. The one under nitrogen was merely unpleasant but not objectionable, whereas the batch under air had a decidedly foul, rotten odour. The gas chromatograms revealed that the air-stored oysters produced methanethiol and dimethyl disulphide which were apparently not produced under nitrogen. Gas chromatography of the vapours from the traps immersed in liquid

nitrogen showed many additional unidentified peaks together with peaks corresponding to hydrogen sulphide, ethanethiol, and 2-methyl-2-propanethiol.

Gas chromatography of the thiols evolved by the regeneration of the mercuric cyanide salts, formed by the vapours from oyster with a foul odour, has verified the presence of the above-mentioned thiols and in addition shows the presence of 2-butanethiol.

The gases in the headspace over freshly canned oysters were sampled according to the technique of Stahl *et al.* (1960). Gas chromatography on a 30% tritolyl phosphate column revealed one peak which had the same retention time as dimethyl sulphide. The volume of sample obtained by this method did not contain enough volatiles for the detection of anything other than dimethyl sulphide.

DISCUSSION

By means of selective precipitation, gas chromatography, and infrared analysis, dimethyl sulphide has been shown to be the main volatile constituent of the aroma of fresh Pacific oysters. The melting point of the mercuric chloride complex was not used as an aid to identification because of its uncertain composition and tendency to decompose.

Decomposition was shown to produce a variety of sulphur-containing compounds, the nature of which depended on whether the oysters were stored in air or nitrogen. These were tentatively identified by gas chromatography as hydrogen sulphide, methanethiol, ethanethiol, dimethyl sulphide, 2-methyl-2-propanethiol, 1-propanethiol, 2-butanethiol, 1-butanethiol, and dimethyl disulphide. Only the air-stored oysters had a rotten odour after approximately 20 hr and presumably this could be attributed to the large amounts of methanethiol and dimethyl disulphide produced under these conditions. It is very likely that the methanethiol resulted from bacterial action and that some of it was oxidized by the air to dimethyl disulphide. Also, a much larger amount of dimethyl sulphide was produced. The presence of carbonyl compounds was very rarely observed in these studies and no attempt was made to identify the trace amounts which occurred.

Aliphatic compounds containing sulphur have been isolated before from marine products. Merritt and Mendelsohn (1962) found dimethyl sulphide in haddock that had been stored for 8 days at 0°C, but they did not find any at the beginning of the storage period. Ackman and Sipos (1964) found dimethyl sulphide to be associated with the "blackberry" problem in cod from the Labrador area. Haas (1935), Obata *et al.* (1951), and Katayama (1958), also found it to be one of the chemical compounds in algae.

Motohiro (1962) identified dimethyl sulphide which was formed from dimethyl-β-propiothetin (DMPT) during the cooking step in the canning process, as the compound responsible for the "petroleum odour" in canned chum salmon. The DMPT is found in relatively large amounts in the Pteropod (*Limacina helicina*) compared to other algae. The chum salmon when it utilizes this algae

as a prime source of food is unable to decompose the DMPT in the liver and retains it in the body, resulting in the "petroleum odour" of the canned product. Challenger *et al.* (1948) have also isolated DMPT from *Polysiphonia fastigiata*, a type of algae.

ACKNOWLEDGMENTS

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REFERENCES

ACKMAN, R. G., AND J. C. SIPOS. 1964. Association of dimethyl sulphide with the "blackberry" problem in cod from the Labrador area. *J. Fish. Res. Bd. Canada*, **21**(2): 423–425.

CHALLENGER, F. 1959. The separation of organic compounds of sulfur by means of mercury salts, p. 16. *In* Aspects of the Organic Chemistry of Sulfur. Academic Press, New York.

CHALLENGER, F., AND M. I. SIMPSON. 1948. Studies on biological methylation. Part 12. Precursor of the dimethyl sulphide evolved by *Polysiphonia fastigiata*. Dimethyl-2-carboxyethyl sulphonium hydroxide and its salts. *J. Chem. Soc.*, 1591.

HAAS, P. 1935. The liberation of methyl sulphide by seaweed. *Biochem. J.*, **29**: 1297.

KATAYAMA, T. 1958. Chemical studies on volatile constituents of seaweed. XIII. On volatile constituents of *Luminaria* sp. *Bull. Japanese Soc. Sci. Fisheries*, **24**(5): 346.

MERRITT, C., AND J. M. MENDELSOHN. 1962. Flavor in fish. Abstracts, Amer. Chem. Soc. Nat'l. Meeting, 142, p. 14A.

MOTOHIRO, T. 1962. Studies on the petroleum odour in canned chum salmon. Mem. Fac. Fish., Hokkaido University.

OBATA, Y., H. IGARASHI AND K. MATANO. 1951. Studies on the flavor of seaweeds (1). On the components of the flavor of some green algae. *Bull. Japanese Soc. Sci. Fisheries*, **17**(2): 60.

STAHL, W. H., W. A. VOELKER AND J. H. SULLIVAN. 1960. A gas chromatographic method for determining gases in the headspace of cans and flexible packages. *Food Technology*, **14**: 14.

YUEH, M. H. 1961. Volatile flavoring constituents of beef and oysters and organoboron compounds of alfalfa. Dissert. Abs., 22, 730.

Characteristics and Nutritional Value of Various Fish Protein Concentrates[1]

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ABSTRACT

Excellent quality fish protein concentrate can be prepared from a variety of raw materials. In this study, the use of whole cod, headed, eviscerated cod, cod trimmings, cod press cake and whole herring was studied as a source of raw material for the production of fish protein concentrate. The process previously described by Power for use with cod fillets was satisfactory for use with all material studied with the exception of herring. Final fat contents were between 0.018–0.056%. For use with herring a modification to the process was required to increase the efficiency of the second extraction. The second extraction, which used a 70:30 isopropanol + water mixture was changed to 99% isopropanol. With this modification a final fat content of 0.17% was achieved. The corrected Protein Efficiency Ratio values for all fish protein concentrates, with the exception of those produced from cod trimming press cake, were higher than that for casein. Lysine values, determined microbiologically were between 9.1 and 15.03% of the protein. The available lysine values were between 6.1 and 10.4% of the protein.

INTRODUCTION

In developing the present process for the manufacture of fish protein concentrate (Power, 1962) the object was to provide a process which would yield the highest possible quality fish protein concentrate. Accordingly the raw material used during development of the process was skinned cod fillets. This material, being essentially muscle, eliminated possible problems which might have resulted from inclusion of bones, skin, and viscera. While the use of cod fillets as a source of starting material for the manufacture of fish protein concentrate results in a high-quality, white product with negligible odour and taste there is a large quantity of lower cost material available to industry with a potential for conversion to fish protein concentrate. For many markets it is not necessary, or in some cases even desirable, that fish protein concentrate be white, of soft texture, or odourless. It is necessary, in all cases, however that the protein be of high nutritional value.

With the above in mind, the present experiment was designed to study the suitability of various materials for conversion to fish protein concentrate using the process previously developed at this Laboratory (Power, 1962). A study was also made of the nutritional quality of the protein of the various protein concentrates produced with this process.

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The starting materials studied were whole cod, headed, eviscerated cod, cod trimmings (eviscerated cod fish from which the fillets had been removed), press cake made from cod trimmings and whole herring. The protein concentrates produced from these sources were evaluated in terms of their physical properties, organoleptic qualities, the Protein Efficiency Ratio (PER) of the protein, and lysine content.

ANALYTICAL METHODS

MOISTURE A.O.A.C. 8th Ed. p. 310 (1955).

COLOUR Samples of compressed protein concentrate were measured for per cent reflectance of light between the wavelengths of 390 and 510 mμ by means of a Beckman model DU spectrophotometer fitted with a diffuse reflectance attachment. A magnesium carbonate block was used as a standard.

FAT For processing studies, 25-g samples were extracted according to the chloroform–methanol phase separation method of Bligh and Dyer (1959) adapted for use with dry materials. The final product was also extracted with ether by the method described in A.O.A.C. 8th Ed. p. 311 (1955).

PROTEIN (NITROGEN × 6.25) A.O.A.C. 8th Ed. p. 12 (1955).

ASH A.O.A.C. 8th Ed. p. 310 (1955).

FIBER A.O.A.C. 8th Ed. p. 370 (1955).

PROTEIN EFFICIENCY RATIO

The method used was that outlined in Official Methods of Analysis of the A.O.A.C. p. 680 (1960) ninth edition. The samples of fish protein concentrate were fed at a 10% protein (N × 6.25) level to rats for comparison with casein.

The samples and the casein standard were incorporated in a synthetic ration on the basis of their proximate composition. The ratios were equalized with respect to moisture, ash, fat, and fiber and were supplemented with vitamin and minerals at levels known to be adequate for the rat.

The prepared diets were fed *ad libitum* to groups of 10 weanling rats each for a period of 4 weeks. Weekly body weights and food consumption records were maintained. At the end of the 4-week period, five animals from each group were killed for a pooled liver fat analysis.

MICROBIOLOGICAL LYSINE

Lysine content was determined by the method of Henderson and Snell (1948).

AVAILABLE LYSINE

Available lysine was determined by the method of Bruno and Carpenter (1957).

VITAMIN ANALYSIS

Thiamine	A.O.A.C. 655 (1960)
Pantothenic Acid	Nielands and Strong, (1948), Arch. of Bio. 19, 2.
Vitamin B-12	Anon. 888 (1960)
Riboflavin	A.O.A.C. 9th Ed. 669 (1960)
Niacin	A.O.A.C. 9th Ed. 667 (1960)
Folic Acid	A.O.A.C. 8th Ed. 830 (1955)
Biotin	Wright and Skegg, (1944), Proc. Soc. Exp. Biol. and Med. 56, 95, (*L. plantarium*).
Pyridoxine HCl	Atkins *et al.* (1943), Ind. and Eng. Chem. Anal. Ed. 15, 141, (*S. carlsbergensis*).

EXPERIMENTAL AND RESULTS

All samples of fish protein concentrate were prepared from 50-lb lots of material from market-size fish (approximately 60 cm in length). The procedure used was that described by Power (1962) and outlined below:

STEP I. The fish material is ground to ¼-inch size in a 1½-hp meat grinder. Sufficient 99% isopropyl alcohol is added to give a 70:30, isopropyl alcohol–water ratio in the mixture making use of the water already contained in the muscle. This requires approximately 19 imp gal of 99% isopropyl per 100 lb of wet material. The mixture is stirred for 15 min in a stainless steel tank, during which time sufficient 20% polyphosphoric acid is added to bring the pH to 5.5. This partly hydrolyzes the connective tissue, making the collagen and gelatin more soluble. The material is dehydrated and slightly denaturated by the alcohol. The texture of the flesh changes from a soft paste to granular particles. This makes possible the use of a high-speed comminutor for further size reduction. The alcohol–fish mixture is then put through a Fitzpatrick comminutor fitted with a screen having ⅛-inch diameter holes.

STEP II. The material from Step I is put in a 30-gal reaction kettle and maintained at 178–180°F with constant agitation for 30 min. A glass reflux condenser is used to prevent loss of alcohol. The material is then pumped to the Bird Basket centrifuge and the liquid removed. The cake then contains 45–50% liquid. The cake is then broken up by passing it through the Fitzpatrick comminutor, this time fitted with a screen having ½-inch-square openings.

STEP III. The shredded cake is replaced in the reaction kettle with 10 gal of 70:30 isopropanol–distilled water mixture for each 100 lb of starting material. The temperature of the mixture is raised to 178–180°F and held there for 15 min with constant agitation. It is then pumped to the Bird centrifuge and the liquid removed. The cake is again shredded in the Fitzpatrick comminutor using a screen with ½-inch-square holes.

STEP IV. The material is then put in the reaction kettle with 99% isopropyl alcohol and again heated to 178–180°F for 15 min while being constantly agitated. In this extraction 10 gal of isopropyl alcohol is used per 100 lb of starting material.

The slurry is then pumped to the centrifuge and the liquid centrifuged off. A further 4 gal of 99% isopropanol is then placed in the kettle, heated to 178–180°F and then pumped into the centrifuge in order to clean the last of the solids from the kettle, pump, and lines and to wash the cake in the centrifuge. The cake is then broken up in the Fitzpatrick comminutor.

The fat content after the extraction is less then .06% (usually from .016 to .04%) on a dry basis as measured by extraction with a methanol–chloroform mixture (Bligh and Dyer, 1959).

STEP V. The ground cake is dried in trays using a cabinet-type dryer in which air at 100–110°F is blown over the trays of material. Drying takes from 24 to 36 hr depending on local weather conditions. The alcohol is removed and the moisture reduced to 3–4%. Finally the concentrate is ground to flour size and packed in polyethylene bags.

EXTRACTION OF FAT AND WATER SOLUBLE MATERIAL

WHOLE COD PROTEIN CONCENTRATE

Live market cod were brought to the Laboratory from Terrence Bay, N.S. They were killed and iced until they had come out of rigor. The fish were then processed to fish protein concentrate by the method outlined above. The process yielded a concentrate of good appearance, the colour was a light fawn, and the texture slightly harder than that of concentrate made from cod fillets. This is probably due to the presence of bone. Odour was negligible and the concentrate was tasteless.

Figure 1 shows the weights of fat and water soluble materials removed from whole cod with each of the three extractions and also the per cent fat

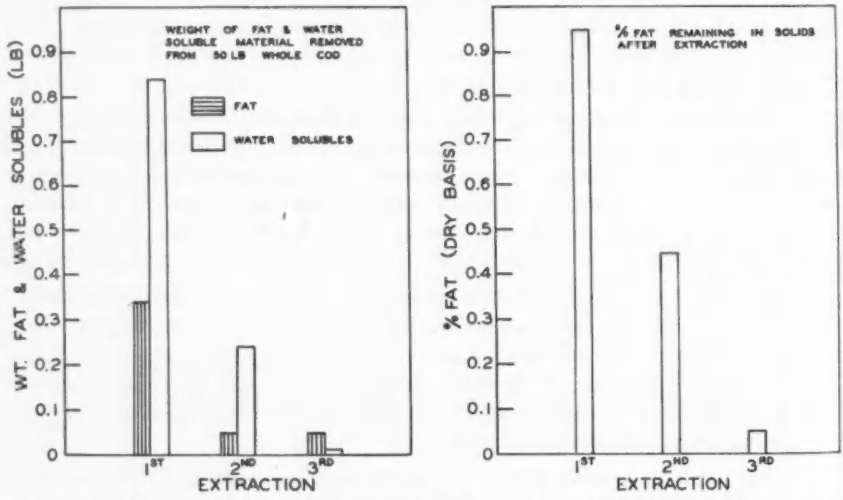

FIG. 1. Weight of fat and water soluble material removed from 50 lb of whole market cod and per cent fat remaining in solids after each extraction.

remaining in the solid material after each extraction. The final product contained 0.056% fat, as determined by chloroform:methanol extraction. This figure is slightly higher than usual, probably because of the presence of the liver.

Headed, Eviscerated Cod

Market cod was obtained live from Terrence Bay, N.S., killed, eviscerated, and the heads removed. They were then iced until they had passed through rigor. Finally they were processed to fish protein concentrate. This concentrate was light fawn in colour, only slightly darker than the concentrate made from whole cod. The reflectivity was 41% of that of a block of magnesium carbonate employing light with a wavelength of 410 mμ. The comparable values for concentrate produced from cod fillets and whole cod are 87% and 42%. The concentrate produced from headed, eviscerated market cod was, for all practical purposes, tasteless and odourless.

The weight of fat and water solubles removed from 50 lb of headed, eviscerated cod is shown in Fig. 2 along with the percentage of fat remaining in the solid material after each extraction. The fat content of the finished concentrate was 0.018%.

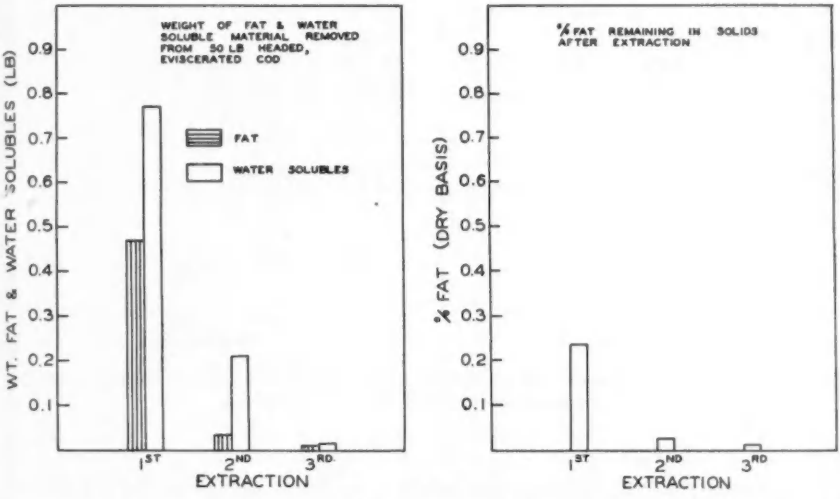

Fig. 2. Weight of fat and water soluble material removed from 50 lb of headed, eviscerated market cod and per cent fat remaining on solids after each extraction.

Cod Trimmings

The average fish plant producing fillets has a large supply of cutting table trimmings left over from the filleting operation. These consist of the remains of the eviscerated round fish after the fillets have been removed from each side. At the present time these are made into fish meal. They are also a potential source of raw material for the manufacture of fish protein concentrate. Fresh

cod trimmings were obtained from a local fish processor and a 50-lb lot was processed to fish protein concentrate. The resulting concentrate was light tan in colour having a reflectivity of 44% at a wavelength of 410 mμ. The final product was essentially tasteless and odourless. Figure 3 shows the weight of fat and water soluble materials removed from 50 lb of cod trimmings along with the per cent fat remaining in the solids after each extraction. Final fat content was 0.027%. Odour and taste were negligible, however the texture was not as soft as that of concentrate produced from fillets due to the presence of bone. The bone content, as determined by flotation in chloroform (Dambergs, unpublished data) was 23.4%. This can be reduced to 7.4% by screening, using 16- and 20-mesh screens, however this process is wasteful of protein as the intermediate and coarse fractions contain only 33 and 29% bones, respectively. The remainder is protein which adheres to the bones.

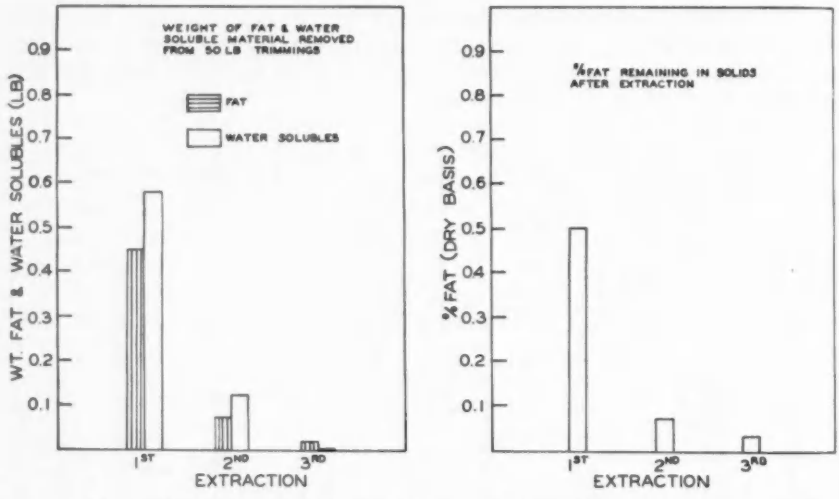

FIG. 3. Weight of fat and water soluble material removed from 50 lb of cod trimmings and per cent fat remaining on solids after each extraction.

COD TRIMMING PRESS CAKE

Press cake is an intermediate product in the manufacture of fishmeal. Fish waste is cooked and pressed to remove as much of the liquid as possible. The liquid is sometimes concentrated and added back to the fishmeal after removal of oil in the case of oily fish.

Fish protein concentrate produced from press cake of cod trimmings equalled that produced from straight trimmings in physical characteristics. Press cake of 60% moisture was produced in the laboratory by two cooking methods. In the first, the trimmings were ground and cooked for 30 min by means of a steam-jacketed kettle. In the second, the ground material was cooked with live steam injected directly into the material. In both cases the cooked material was then

pressed to 60% moisture in a hydraulic press. The press juice was saved for analysis and the cake processed by the regular method to concentrate. Although the concentrate produced was of good physical appearance, preliminary analysis of the press juice showed it to contain 9% solids, all or nearly all nitrogenous material of nutritional value. These solids represent a considerable percentage of the total solids available, e.g. from 30 lb raw material containing

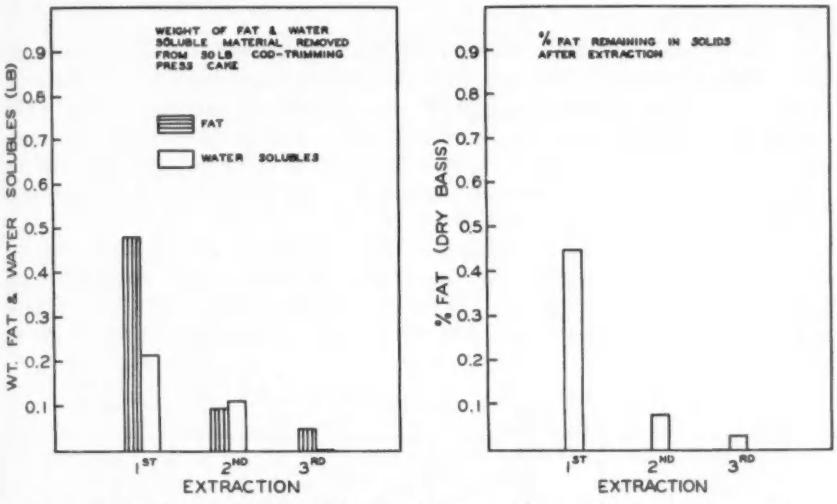

FIG. 4. Weight of fat and water soluble material removed from 50 lb of indirectly cooked cod trimming press cake and per cent fat remaining in solids after each extraction.

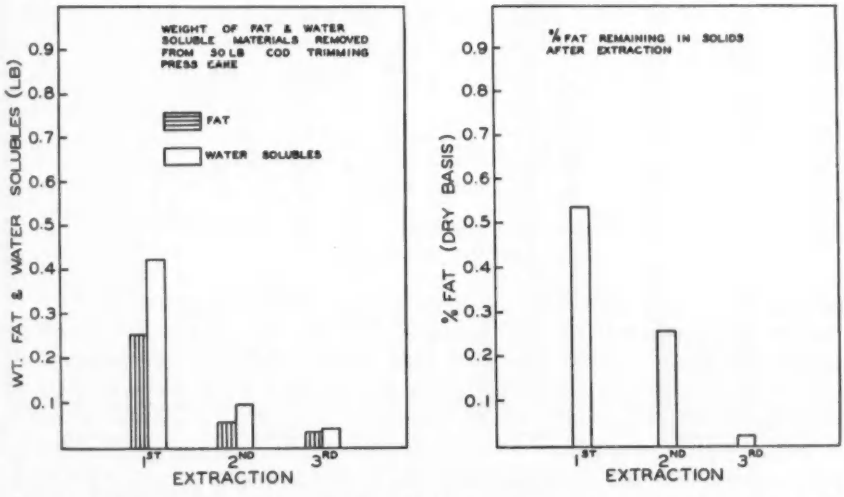

FIG. 5. Weight of fat and water soluble material removed from 50 lb of cod trimming press cake cooked with live steam and per cent fat remaining in solids after each extraction.

approximately 10 lb of solids, 1.99 lb of solids were contained in the press juice or approximately 20% of the total solids.

Extraction data for protein concentrate made from indirectly cooked cod trimming press cake are given in Fig. 4. Figure 5 presents the data for cod trimming press cake cooked with live steam. The residual fat in these protein concentrates was 0.035% and 0.032%, respectively.

Whole Herring

The standard procedure was tried with whole herring as the raw material. The results were not satisfactory as the extraction was inefficient and the final product contained a higher fat content than is desirable (average 1.1%). The extraction data determined by Dambergs (1959) was specifically for the phospholipids found in the flesh of non-oily fish such as cod and haddock. As the fat in herring is almost entirely triglycerides, the extraction procedure was modified to give better extraction of triglycerides.

It was found that by changing the second extraction from a 70:30 mixture of isopropanol–water to 99% isopropanol the efficiency of the extraction was improved to the point where the final product contained only 0.18% fat. Figure 6 shows the results of extraction with the standard procedure and the modified procedure.

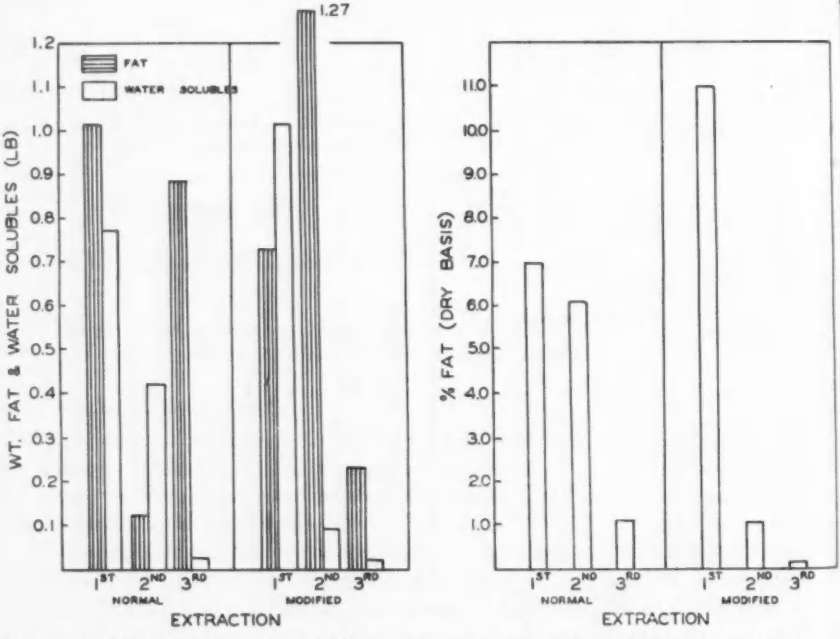

Fig. 6. Weight of fat and water soluble material removed from 50 lb of whole herring by both the standard process and the modified extraction procedure and per cent fat remaining in solids after each extraction.

Whole herring produced a slightly darker concentrate than the other materials in this group. Its reflectivity with light of 410 mμ wavelength is 28%. This concentrate has negligible odour and a very slight, not unpleasant taste.

The per cent reflectivity for all concentrates with light of from 390 to 480 mμ wavelength is shown in Figure 7. The standard against which these samples were compared was a block of magnesium carbonate which was assumed to have a reflectivity of 100%.

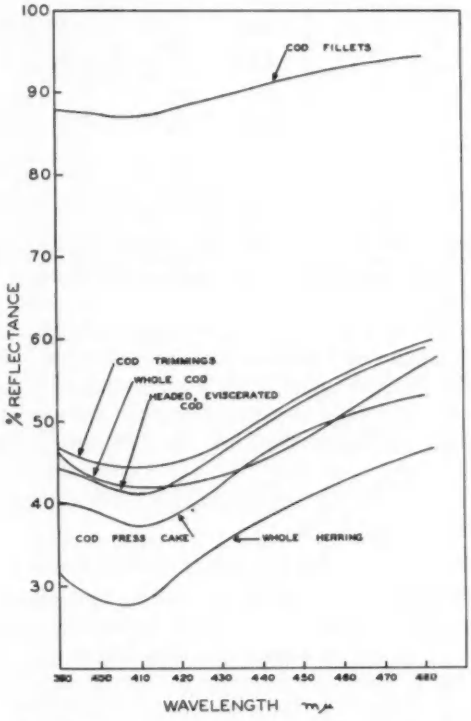

FIG. 7. Diffuse reflectance curves for various fish protein concentrates.

The proximate composition of the various samples of fish protein concentrate is shown in Table I. The samples produced from cod fillets, whole cod, headed, eviscerated cod, and whole herring have the highest levels of protein while the samples produced from press cake are lower due to the loss of protein during the pressing operation. The protein content of fish protein concentrate samples depends to some extent on the size of fish used as starting material. This is especially true of samples produced from trimmings where an effort has been made to remove the maximum amount of muscle during the filleting operation. Protein concentrate made from trimming of market cod approximately 60 cm in

TABLE I. Proximate analysis of fish protein concentrates.

Raw material	Percentage protein (dry basis)	Moisture (%)	Ash (%)	Fiber (%)	Fat (%) (ether extract)	Fat (%) (chloroform–methanol extraction)
Cod fillets	92.9	4.64	1.89	0.50	0.02	0.033
Whole cod	84.7	7.62	14.6	0.88	0.02	0.056
Headed eviscerated cod	90.26	5.25	8.37	0.81	0.02	0.02
Cod trimmings	87.2	3.54	11.42	0.34	0.039	0.04
Cod trimming[a] press cake	76.6	4.19	26.68	0.63	0.03	0.035
Cod trimming[b] press cake	70.7	5.66	23.78	0.60	0.01	0.032
Whole herring	89.7	8.24	7.13	0.94	0.09	0.18

[a]Cod trimmings cooked with indirect heat and pressed to 60% moisture.
[b]Cod trimmings cooked with live steam and pressed to 60% moisture.

length showed a protein content of 87.2% while samples produced from the trimmings of much larger fish showed a protein content of 73.6% on a dry basis probably due to the increased size of the bone structure. Ash content of the samples depends mainly on the amount of bone in the raw material. Protein concentrate produced from fillets had less than 2% ash while for concentrate produced from press cake it is over 26%. In all samples the residual fat was less than 0.1% with the exception of the sample made from whole herring. In this case the fat content was 0.18%.

NUTRITIONAL PROPERTIES OF FISH PROTEIN CONCENTRATES

In order to assess the nutritional quality of the various fish protein concentrates, feeding tests were conducted with rats to determine the PER and the amount of lysine and available lysine was determined. In addition to those tests, vitamin determinations were made on the concentrate produced from cod fillets.

Table II gives the vitamin analysis of fish protein concentrate as produced by the regular procedure and as produced by the regular procedure with an additional extraction with the 70:30 isopropanol:water mixture. One additional extraction does not remove additional quantities of the vitamins with the exception of niacin which is lowered by approximately 50%. Although these values are not as low as those for low-vitamin casein, they are low enough to permit the use of this protein concentrate in place of "vitamin free" casein for certain vitamin deficiency tests.

To assess the nutritive value of the protein of the various fish protein concentrates, the PER's were determined by incorporating the protein in the diets of rats at the 10% level. The PER's were adjusted to a value of 2.50 for casein. The values for the PER's are shown in Table III and Figure 8. The corrected

TABLE II. Vitamin analysis of fish protein concentrate made from cod fillets.

Vitamin	Regular extraction procedure	Regular extraction plus one additional extraction with 70:30 isopropanol:water solution
Thiamine HCl	0.76	0.73
Pantothenic acid	2.94	3.03
Vitamin B-12	0.017	0.016
Riboflavin	1.30	1.36
Niacin	2.62	1.28
Folic acid	0.276	0.486
Biotin	0.110	0.124
Pyridoxine HCl	5.40	5.32

TABLE III. Nutritional value of fish protein concentrates fed at the 10% protein level for 4 weeks to a group of 10 weanling rats.

	Source of raw material for fish protein concentrate						
	Cod fillets	Whole cod fish	Headed, eviscerated cod	Cod trimmings	Cod trimming press cake[a]	Cod trimming press cake[b]	Whole herring[c]
Initial wt. (g)	49	49	49	47	49	49	50
1-week wt. (g)	74	73	69	65	59	60	68
2-week wt. (g)	103	100	101	98	74	79	99
3-week wt. (g)	133	122	114	126	90	99	118
4-week wt. (g)	166	158	149	158	111	123	152
Weight gain (g)	117	110	100	111	62	74	103
Food (g) consumed	379	398	374	383	271	337	352
PER	3.09	2.75	2.68	2.91	2.28	2.20	3.21
Corrected[e] PER	2.97	2.64	2.58	2.57	2.19	2.12	2.74
Liver fat[d] (% dry basis)	7.86	10.2	7.33	7.84	8.42	9.13	9.05

[a]Cod trimmings cooked with indirect heat and pressed to 60% moisture.
[b]Cod trimmings cooked with live steam and pressed to 60% moisture.
[e]Protein efficiency ratio corrected to casein value of 2.50.
[d]Average liver fat for casein fed rats — 10.9% (dry basis).
[c]Mixed immature herring 15–20 cm in length, average weight of gonads: male 2.2%, female 3.3%.

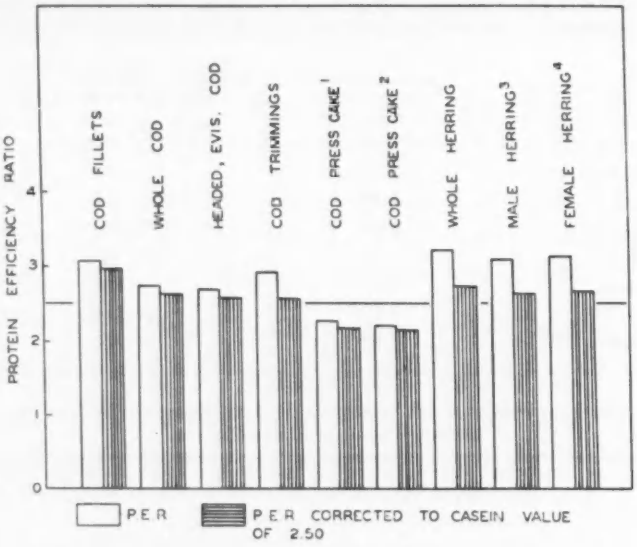

FIG. 8. Protein efficiency ratios for protein of various fish protein concentrates.

[1]Cod trimmings cooked with indirect heat and pressed to 60% moisture.
[2]Cod trimmings cooked with live steam and pressed to 60% moisture.
[3]Male herring just prior to spawning; gonads 19.5% of total weight.
[4]Female herring just prior to spawning; gonads 22% of total weight.

PER's of all samples are higher than the corresponding values for casein with the exception of the samples produced from press cake. Again the loss of proteinaceous material during pressing is the most probable cause of the decrease in quality of the protein of this material. The press cake cooked by the use of live steam, directly injected into the material, showed the lowest PER due to the extractive effect of the steam condensate. The highest PER's were given by the protein in the concentrate produced from cod fillets, whole herring, and whole cod. The PER's for these concentrates were 2.97, 2.74, and 2.64, respectively. Protein concentrate made from headed, eviscerated cod and cod trimmings gave intermediate values for the PER; 2.58, and 2.57, respectively. These can be compared with a value of 2.50 for casein. The samples of protein concentrate produced from cod trimming press cake gave the lowest values for the PER; 2.19 and 2.12 considerably lower than casein.

Samples of fish protein concentrate were also produced from mature male and female herring just prior to spawning. The gonads of these fish made up 19.5% and 22.0% of the total body weight. Table IV shows the results of biological evaluation of the protein concentrate from these sources. Both samples show PER's better than casein, the PER of the male herring being 2.64 and of the female herring 2.67.

TABLE IV. Nutritional value of fish protein concentrates, made from male and female herring just prior to spawning, fed at the 10% protein level for 4 weeks to groups of 10 weanling rats.

	Source of raw material for fish protein concentrate	
	Male Herring[a]	Female Herring[b]
Initial wt. (g)	50	50
1-week wt. (g)	66	67
2-week wt. (g)	96	97
3-week wt. (g)	115	117
4-week wt. (g)	149	150
Total gain (g)	99	101
Total food consumption (g)	351	354
PER	3.10	3.13
Corrected PER	2.64	2.67
Liver fat[c] (% dry basis)	10.5	8.53

[a]Whole male herring; gonads 19.5% of total weight.
[b]Whole female herring; gonads 22% of total weight.
[c]Average liver fat for casein fed rats — 10.9% (dry basis).

Litwack *et al.* (1952) have shown that one of the factors resulting in deposition of lipids in the liver of experimental animals is the quality and quantity of the protein in the ration. The addition of 6% casein to a diet was sufficient to reduce the average liver lipid from 29% to 10.2%. Proteins and amino acids supplied by whole protein such as casein appear to be able to control the deposition of fat in the liver.

The liver fats of rats fed diets containing the protein of the various fish protein concentrates were, in all cases, lower than the liver lipids of the animals on the casein diet. As shown on Tables III, IV and Fig. 9, the liver lipids of the animals fed the fish protein concentrate diets were between 7.84% and 10.53%. Rats fed the diet containing casein had an average liver lipid value of 10.9%.

Morrison and Campbell (1960) have shown that the PER value for diets fed to rats is a direct function of the percentage lysine in the protein. The lysine content of the proteins of the various fish protein concentrates tested is relatively high having values of between 9.1% of the protein and 15.03% of the protein as shown in Table V. Guttman and Vandenheuvel (1957) found the lysine content of fish protein concentrate made from cod and haddock trimmings to be 11.48% when determined by means of ion-exchange chromatography.

The quality of the protein in mixed diets for animals or man is often limited by its lysine content (Carpenter, 1960). The draft specification for fish protein concentrate, proposed by the Food and Agriculture Organization of the United Nations at the F.A.O. International Conference on Fish in Nutrition in Washington, D.C. in 1961, suggests a minimum available lysine content of 6.5% of the protein.

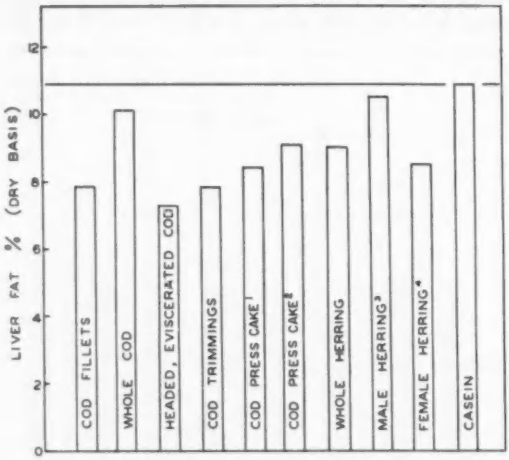

FIG. 9. Percentage of lipids in the liver of rats fed diets containing casein and fish protein concentrates.

[1]Cod trimmings cooked with indirect heat and pressed to 60% moisture.
[2]Cod trimmings cooked with live steam and pressed to 60% moisture.
[3]Male herring just prior to spawning; gonads 19.5% of total weight.
[4]Female herring just prior to spawning; gonads 22% of total weight.

TABLE V. Lysine and available lysine content of fish protein concentrate.

	Lysine (%)	Lysine (% of protein)	Available lysine	Available lysine (% of protein)
Cod fillets	12.6	14.2	8.49	9.58
Whole cod fish	11.5	14.7	7.87	10.1
Headed, eviscerated cod	11.7	13.3	7.04	8.23
Cod trimmings	7.7	9.1	7.5	8.9
Cod trimming press cake[a]	10.1	13.8	5.36	7.31
Cod trimming press cake[b]	9.46	14.2	6.93	10.4
Herring	11.6	14.1	5.82	7.07
Male herring[c]	11.2	15.03	6.28	8.43
Female herring[d]	9.63	11.3	5.13	6.14

[a]Cod trimmings cooked with indirect heat and pressed to 60% moisture.
[b]Cod trimmings cooked with live steam and pressed to 60% moisture.
[c]Male herring just prior to spawning; gonads 19.5% of total weight.
[d]Female herring just prior to spawning; gonads 22% of total weight.

The values for available lysine are shown in Table V. All samples show available lysine values of between 6.14% of the protein and 10.4% of the protein. With the exception of the protein prepared from mature female herring just prior to spawning, all values are above the minimum value of 6.5% recommended by F.A.O.

SUMMARY

The improved procedure for the manufacture of fish protein concentrate (Power, 1962) has been shown to be satisfactory for producing a high-quality protein concentrate from cod fillets, whole cod, headed, eviscerated cod and cod trimmings. The fish protein concentrate thus produced has a high PER, satisfactory colour, odour, taste, and texture. Lysine and available lysine values are relatively high.

With a modification to the extraction procedure an excellent product was also produced from whole herring. Fish protein concentrates of lower nutritional value were produced from cod trimming press cake.

ACKNOWLEDGMENTS

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REFERENCES

ANON. 1960. The United States Pharmacopoeia, **16**: 888.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1955. Official methods of analysis. 8th ed.

1960. Official methods of analysis. 9th ed.

ATKINS, L., A. S. SHULTZ, W. L. WILLIAMS AND C. N. FREY. 1943. Yeast microbiological methods for determination of vitamins. Pyridoxine. *Ind. Eng. Chem. Anal. Ed.*, **15**: 141–144.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.*, **37**: 911–917.

BRUNO, D., AND K. J. CARPENTER. 1957. A modified procedure for the estimation of available lysine in food proteins. *Biochem. J.*, **67**: 13.

CARPENTER, K. J. 1960. The estimation of the available lysine in animal protein foods. *Biochem. J.*, **77**: 604–610.

DAMBERGS, N. 1959. Extractives of fish muscle. 2. Solvent–water ratio in extraction of fat and water-solubles. *J. Fish. Res. Bd. Canada*, **16**(1): 63–71.

GUTTMANN, A., AND F. A. VANDENHEUVEL. 1957. The production of edible fish protein ("fish flour") from cod and haddock. *Fish. Res. Bd. Canada, Atlantic Prog. Rept.*, No. 67, pp. 29–31.

HENDERSON, L. M., AND E. E. SNELL. 1948. A uniform medium for determination of amino acids with various organisms. *J. Biol. Chem.*, **172**: 15–29.

LITWACK, G., L. V. HANKES AND C. A. ELVEHJEM. 1952. Effect of factors other than choline on liver fat deposition. *Proc. Soc. Exp. Biol. Med.*, **81**: 441.

MORRISON, A. B., AND J. A. CAMPBELL. 1960. Studies on the nutritional value of defatted fish flour. *Canadian J. Biochem. Physiol.*, **38**: 467–473.

NIELANDS, J. B., AND F. M. STRONG. 1948. The enzymatic liberation of pantothenic acid. *Arch. Biochem.*, **19**: 287–291.

POWER, H. E. 1962. An improved method for the preparation of fish protein concentrates from cod. *J. Fish. Res. Bd. Canada*, **19**(6): 1039–1045.

WRIGHT, L. D., AND H. R. SKEGGS. 1944. The determination of biotin with *Lactobacillus arabinosus*. *Proc. Soc. Exp. Biol. Med.*, **56**: 95–98.

Ascidians of the Canadian Arctic Waters[1]

By Winona Bethune Trason[2]

ABSTRACT

The ascidians of the Canadian Arctic collected by the Eastern Arctic Investigations and Arctic Unit of the Fisheries Research Board of Canada from the years 1947 to 1962 inclusive have been identified and the numbers collected tabulated. Information relevant to collecting stations is presented in tables and on maps.

The ascidians which are the subject of this report were collected by expeditions conducted by the Fisheries Research Board of Canada, Eastern Arctic Investigations, during the years 1947 to 1955 inclusive, and by the Fisheries Research Board of Canada Arctic Unit during the years 1956 through 1962.

The specimens were taken at various points in Canadian arctic waters from Hudson Bay in the east through Mackenzie Bay in the west (see Fig. 1–5). Lists of data relevant to all collection stations made by the Fisheries Research Board of Canada for the years 1947 through 1958 have been published previously by Dunbar and Grainger (1952), Grainger (1954), Grainger and Dunbar (1956), and Grainger and Hunter (1959). Data pertinent to all stations at which ascidians were collected from 1947 through 1962 have been included in Tables I and II and in Fig. 1–5 in this paper.

Arctic ascidians are relatively well known from reports by Arnback (1922–1934), Hartmeyer (1923–24), Redikorzev (1907, 1907a, 1910), and others. Information from these authors relative to the ascidians of the North American arctic has been brought together by Van Name (1945).

Among the approximately 1480 specimens collected, 27 species were identified. Of these, three species made up almost 50% of the collections: *Boltenia ovifera* — 253 specimens, *Boltenia echinata* — 250 specimens, and *Ascidia callosa* — 230 specimens. The number of each species taken at each collection station has been recorded and presented in Tables III and IV. Where definite species assignments have not been made, the specimen was either in a poor state of preservation, was a juvenile, or was a member of a genus in which the taxonomy is not clear enough to make such assignations.

The species found are listed below and each name is followed by a numeral in brackets indicating the total number taken from 1947 through 1962.

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TABLE I. List of stations, 1947–1959.

Station number	Map location	North latitude	West longitude	Depth (m)	Collecting gear	Bottom
1947						
3	S Ungava Bay	58°50′	68°13′	29	dredge	stones
18	W Ungava Bay	60°02′	69°03′	84	dredge	mud and stones
20	W Ungava Bay	59°53′	69°29′	64	dredge	sand and mud
21	W Ungava Bay	59°54′	69°31′	64	dredge	sand and mud
28	SW Ungava Bay	58°54.5′	68°59′	27	dredge	–
30	S Ungava Bay	58°42.4′	68°10′	37	dredge	–
33	E Ungava Bay	59°13′	65°45′	27	dredge	sand with small stones
45	NE Ungava Bay	60°24.5′	64°50.5′	27–37	dredge	mud and stones
1948						
53	S Ungava Bay	58°38′	68°13′	9	dredge	–
Tunnutaksuk	NE Ungava Bay			–	–	–
1949						
102	NE Ungava Bay	60°23.5′	64°52′	90–130	beam trawl	–
					dredge	glacial mud
103	NE Ungava Bay	60°24′	64°58′	146	beam trawl	–
				164–255	dredge	rock
				128	stramin net	–
107	NE Ungava Bay	60°22′	64°47′	55–73	dredge	rocks, stones, stagnant mud
126	W Ungava Bay	60°04′	69°26′	66–73	dredge	–
1950						
203	S Ungava Bay	58°50′	68°18′	30	dredge	–
209	Cape Hopes Advance	61°05.3′	69°34′	82	dredge	rock
210	Cape Hopes Advance	61°04.5′	69°39′	91–109	dredge	rock
217	S Hudson Strait	61°41′	71°56′	intertidal	–	–
225	N Cape Chidley	60°40.5′	64°39.7′	Jigged from bottom	–	rock
226	N Cape Chidley	60°38′	64°38.9′	90–100	dredge	–
232	E Ungava Bay	59°23′	65°01′	10	–	–
238	S Resolution Island	61°20′	64°53′	18–36	benthos collecting	–
1951						
317	SE Frobisher Bay	62°44′	65°29′	27–36	dredge	silt, stones
318	SE Frobisher Bay	62°43′	65°38′	55	dredge	–
331	SE Frobisher Bay	62°42′	65°31′	77	trawl	–
333	SE Frobisher Bay	62°39′	65°47.5′	104–137	trawl	–
334	SE Frobisher Bay	62°38′	65°52′	183–192	trawl	–
1952						
404	SE Frobisher Bay	62°48.′5	65°28′	13	jig	–
406	SE Frobisher Bay	62°74′	65°26.5′	46	dredge	–
413	NE Cumberland Sound	66°12.5′	66°24′	26	dredge	thick mud
419	SW Frobisher Bay	62°15.5′	66°18′	13	jig and anchor	–
1953						
502	W Hudson Bay	58°50′	94°04′	27	beam trawl	–
534	SW Baffin Island	64°14′	76°33′	12	anchor and jig	–
541	SW Baffin Island	64°25′	78°05′	65–74	plankton net	–
542	SW Baffin Island	64°24′	77°56′	0–38	dredge	rock, dark mud
547	SE Southampton Island	63°41′	80°12′	73	dredge	rocks, small stones
549	S Southampton Island	63°36′	82°00′	73–75	dredge	small rocks
553	W Hudson Bay	62°09′	92°25′	36–54	beam trawl	–
1954						
607	S Coats Island	62°13′	83°24′	14	dredge	gravel, rock
614	NE Coats Island	62°58.5′	82°41′	13–18	dredge	fine sand
616	N Coats Island	62°15.5′	83°41′	85–90	dredge	small stones
1955						
713	W Foxe Basin	66°55′	81°20′	25–33	dredge	rock
715	N Fury and Hecla Strait	69°34	80°17′	14–15	dredge	mud and fine sand
720	N Melville Peninsula	69°21.5′	81°40.5′	2–7	plankton net	–
722	N Melville Peninsula	69°20′	82°00′	36–45	dredge	mud
723	N Melville Peninsula	69°22.7′	81°44.3′	22	plankton net	–
1956						
719	N Melville Peninsula	69°20.5′	81°43.5′	50	dredge	mud, small stones
723	N Melville Peninsula	69°22.7′	81°44.3′	20	dredge	thick mud
811	Fury and Hecla Strait	69°37.2′	81°06.3′	110	dredge	rock
817	N Fury and Hecla Strait	69°36′	80°13′	20	beam trawl	–
1957						
901	N Foxe Basin	ca. 69°06′	ca. 79°11′	28	VanVeen grab	clay
				15	VanVeen grab	sandy mud stagnant mud
				45	dredge	rocks, mud
				ca. 25	dredge	rock
				ca. 20	dredge	rock

TABLE I. (Continued)

Station number	Map location	North latitude	West longitude	Depth (m)	Collecting gear	Bottom
1958						
58-21	NE Hudson Bay	62°36′	78°16′	53	dredge	rock
58-32	E Hudson Bay	57°31′	79°41′	12	VanVeen grab	–
1959						
59-11	James Bay	52°17′	78°50′	22	beam trawl	mud, small stones
59-15	James Bay	53°26′	79°52′	55–60	dredge	mud, small stones
59-20	SE Hudson Bay	56°27′	78°31′	50	dredge	rock
59-22	SE Hudson Bay	56°32′	77°27′	38	stramin net	rock and mud
59-26	SE Hudson Bay	55°23′	77°40′	90	beam trawl	mud
59-28	SE Hudson Bay	55°59.7′	79°52.8′	10–15	dredge	–
59-29	SE Hudson Bay	55°50′	80°15′	40	dredge	rock
59-38	SE Hudson Bay	56°09′	76°26′	95–130	beam trawl	mud
59-50	SE Hudson Bay	58°00′	79°38′	50–60	dredge	rock
59-51	SE Hudson Bay	57°02′	79°41′	80–94	dredge	rock, mud, sand
59-52	SE Hudson Bay	56°32′	79°02.5′	47	dredge	rock
59-64	SE Hudson Bay	55°13′	82°08′	26	dredge	gravel
			C.G.S. Labrador Collection			
57	NE Hudson Bay	62°03′	83°00′	68	–	specimens attached to rocks

ORDER APLOUSOBRANCHIA

Family SYNOICIDAE

Aplidium glabrum Verrill (89)
Synoicum pulmonaria (Ellis and Solander) (11)
Synoicum sp. (92)

Family DIDEMNIDAE

Didemnum albidum (Verrill) (4)
Lissoclinum aureum Verrill (2).
Unidentified didemnids (4)

Family POLYCITORIDAE

Polycitor vitreus (Sars) (10)
Distaplia clavata (Sars) (12)

ORDER PHLEBOBRANCHIA

Family ASCIDIIDAE

Ascidia callosa Stimpson (230)
Ascidia obliqua Alder (4)
Ascidia prunum O. F. Mueller (10)
Ascidia sp. (8)

Family RHODOSOMATIDAE

Chelyosoma macleayanum Broderip and Sowerby (1)

ORDER STOLIDOBRANCHIA

Family BOTRYLLIDAE

Botrylloides aureum Sars (18)

Family STYELIDAE

Kukenthalia borealis (Boldschaldt) (15)
Cnemidocarpa finmarkiensis (Kiaer) (4)
Cnemidocarpa rhizopus (Redikorzev) (9)
Dendrodoa aggregata (Rathke) (79)
Dendrodoa pulchella (Verrill) (2)
Dendrodoa grossularia (Van Beneden) (2)
Dendrodoa sp. (4)
Styela coriacea (Alder and Hancock) (23)
Styela rustica (Linnaeus) (66)
Pelonaia corrugata Goodsir and Forbes (6)

TABLE II. List of stations, 1960, 1961, 1962.

Station number	Map location	North latitude	West longitude	Depth (*m*)	Bottom
1960					
60-1001	E Mackenzie Bay	69°32.5′	138°55′	12–37	mud
60-1008	E Mackenzie Bay	69°33.8′	138°55′	5–11	mud
60-1011	E Mackenzie Bay	69°34.2′	138°54.3′	2–4	mud
60-1012	E Mackenzie Bay	69°34′	138°54′	4	mud
60-1016	E Mackenzie Bay	69°37′	139°57′	11–13	gravel
60-1025	E Mackenzie Bay	69°33′	138°55′	3.5	mud
60-1035	E Mackenzie Bay	69°32′	138°57′	21–44	mud
60-1039	E Mackenzie Bay	69°34.6′	138°57.4′	4	mud
1961					
61-1021	Liverpool Bay	69°56′	129°37′	11	mud, sand
61-1026	Liverpool Bay	69°35.5′	131°02′	55	sand, rock
61-1053	Liverpool Bay	69°27.5′	131°45′	9	sand
61-1082	NW Mackenzie Bay	70°13′	132°36′	34	mud
61-1	W Hudson Bay	60°24′	93°55′	60	rock, mud
61-2	W Hudson Bay	61°00′	93°49′	45	rock
61-3	W Hudson Bay	61°05′	93°54′	18–20	rock
61-4	W Hudson Bay	61°52′	92°20′	40	rock
61-5	W Hudson Bay	62°30′	91°05′	70	rock
61-10	NW Hudson Bay	63°43′	91°43′	80 75–90	rock
61-11	NW Hudson Bay	63°39′	91°27′	20–70	rock
61-12	NW Hudson Bay	63°33′	91°02′	90–100 50–60	rock
61-13	NW Hudson Bay	63°21′	90°37′	95–100	rock
61-15	NW Hudson Bay	63°52′	88°37′	45	rock
61-18	NW Hudson Bay	63°37′	87°33′	49	rock
61-21	NW Hudson Bay	64°43′	86°57′	80	rock
61-24	NW Hudson Bay	65°07′	86°45′	44–46	rock
61-26	NW Hudson Bay	65°24′	86°44′	75	rock
61-30	NW Hudson Bay	65°59′	85°45′	57	rock
61-32	E Foxe Basin	66°11′	84°12′	145	rock, mud
61-39	E Foxe Basin	65°32′	84°52′	42	rock, mud
61-43	E Foxe Basin	66°14′	84°49′	275	rock
1962					
62-006	Liverpool Bay	69°49.3′	130°19′	2–18	sand, mud
62-008	Liverpool Bay	69°49.3′	130°19′	5–15	sand, mud, rock
62-032	Liverpool Bay	69°48′	130°19′	10	sand, mud
62-2003	E Prince Regent Inlet	72°45.5′	94°07′	34–35	gravel
62-2008	E Prince Regent Inlet	72°45.5′	94°04.5′	30–45	sand
62-2011	E Prince Regent Inlet	72°45.3′	94°04′	40	sand
62-2012	E Prince Regent Inlet	72°44.6′	94°05′	36	clay, mud
62-2014	E Prince Regent Inlet	72°44.1′	94°06′	35	clay
62-2031	E Prince Regent Inlet	72°46.5′	94°13′	40	clay, mud
62-2032	E Prince Regent Inlet	72°46.5′	94°15.5′	59–62	–
62-3001	W Ellesmere Island	80°00′	86°00′	16 5–30	mud
62-4001	N Barrow Strait	74°36′	94°13′	35, 50	–
62-4006	N Barrow Strait	74°39.1′	94°15.7′	15	clay
62-4009	N Barrow Strait	74°37.5′	94°26.3′	10	Laminaria

Family PYURIDAE

Boltenia ovifera (Linnaeus) (253)
Boltenia echinata (Linnaeus) (250)
Halocynthia pyriformis (Rathke) (16)

Family MOLGULIDAE

Molgula griffithsii (MacLeay) (75)
Molgula siphonalis (Sars) (14)
Molgula retortiformis (Verrill) (2)
Rhizomolgula globularis (Pallas) (54)
Unidentified molgulids (9)

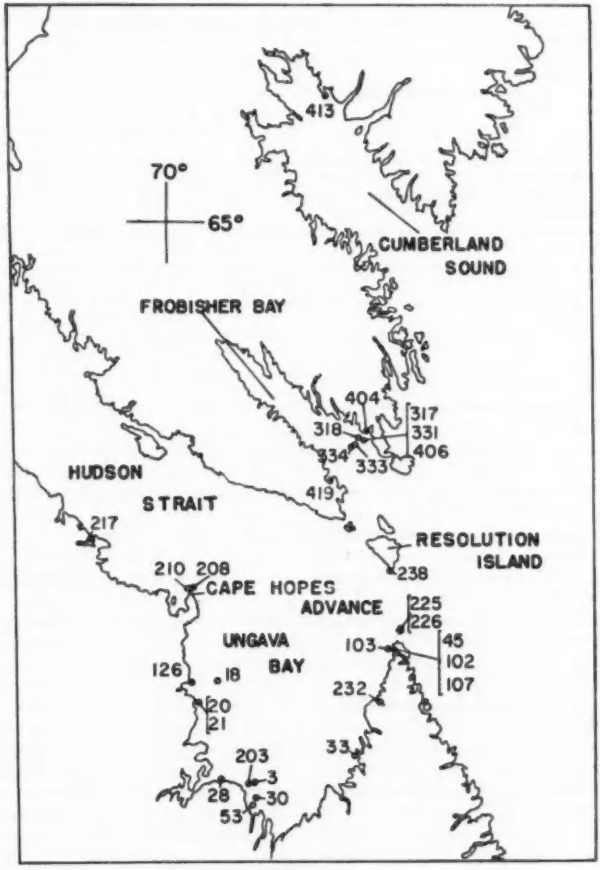

FIG. 1

TABLE III.

Sta. No.	Aplidium glabrum	Synoicum pulmonaria	Synoicum sp.	Didemnum albidum	Lissoclinum aureum	Unidentified didemnida	Distaplia clavata	Polycitor viticeus	Arctica callosa	Arctica obliqua	Arctica prunum	Arctica sp.	Botryllodes aureum	Kukenthalia borealis	Chemilocarpa hancockensis	Chemilocarpa rhizogyra	Dendrodoa asperopata	Dendrodoa groenlandica	Dendrodoa sp.	Styela cortiacea	Styela rustica	Pelonaia corrugata	Holozoa arctica	Holozoa echinata	Halocynthia pyriformis	Molgula griffithsii	Molgula siphonalis	Molgula tectiformis	Rhizomolgula globularis	Unidentified molgulids
3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	7	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
28	72	-	72	1	-	-	-	5	-	-	-	2	2	-	2	-	-	2	-	-	-	-	-	-	-	1	-	-	-	-
30	-	-	17	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	1	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-
45	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	57	3	-	-	-	-	-	-
53	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	3	-	-	-	-	-	-	-
Tun.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	44	-	-	-	-	-	-	-
103	2	-	-	-	-	-	-	1	4	-	-	-	-	9	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
107	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	-	-	-	1	-
126	-	-	-	-	-	-	-	-	6	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
203	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	1	1	-	-	-	-	-	1	3	-	-	-	-	-	-
208	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	2	1	1	-	-	-	-	-
210	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
217	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
225	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	31	-	-	-	1	-	-	-
226	-	3	-	-	-	-	4	-	34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
232	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
238	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
317	11	-	3	1	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-
318	3	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
331	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-
333	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-
334	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	1	-	-	-	1	-	-	1	-	-	-	-	-	-
404	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE III. (Continued)

TABLE III. (Continued)

Sta. No.	Aplidium glabrum	Synoicum pulmonaria	Synoicum sp.	Didemnum albidum	Leptoclinum gyrosum	Unidentified didemnids	Distaplia clavata	Polycitor vitreus	Ascidia callosa	Ascidia obliqua	Ascidia prunum	Ascidia sp.	Botryllodes gyrosum	Kukenthalia borealis	Chemidocarpa finmarkiensis	Chemidocarpa rhizopus	Dendrodoa aggregata	Dendrodoa grossularia	Dendrodoa sp.	Styela coriacea	Styela rustica	Polycarpa cartilaginea	Boltenia echinata	Boltenia ovifera	Boltenia echinata	Halocynthia pyriformis	Molgula griffithsii	Molgula strphonalis	Molgula retortiformis	Rhizomolgula globularis	Unidentified molgulids
406	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	1	-	-	1	-	-	2	-	-	-	-	-
413	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
410	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-	1	-	-
602	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
534	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	3	1	-	-	-	1	-	-	-	-
541	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	1	-	-	2	-	-	-	-	-
542	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
547	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
549	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
553	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
607	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
614	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	22	-	-	-	-	1	-
616	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	2	-	-	2	-	-	-	1	42	2	-	-	-	-	-
713	-	-	-	-	-	1	-	-	4	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	1	-	8	-
715	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	-	-	-	-	-	-
720	-	-	-	-	-	-	-	-	6	-	3	-	-	-	-	-	-	-	-	-	8	-	-	-	5	-	-	1	-	-	-
722	-	-	-	-	-	-	-	-	47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
728	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-
719	-	-	-	-	-	-	-	-	67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-	-
723	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
811	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	58	-	-	-	-	-	-
817	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	1	3	-	-	-
901	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
58-21	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58-32	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C.G.S. Labrador 57	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE IV.

Sta. No.	*Aplidium glabrum*	*Synoicum pulmonaria*	*Didemnum albidum*	Undetermined didemnids	*Polycitor rubrus*	*Astidia callosa*	*Astidia obliqua*	*Astidia prunum*	*Chelyosoma macleayanum*	*Boltyllioides aureum*	*Chemidocarpa finmarkiensis*	*Chemidocarpa rhizopus*	*Dendrodoa aggregata*	*Dendrodoa pulchella*	*Styela coriacea*	*Styela rustica*	*Boltenia ovifera*	*Boltenia echinata*	*Halocynthia pyriformis*	*Molgula griffithsii*	*Molgula siphonalis*	*Rhizomolgula globularis*	Unidentified molgulids
59-11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	3
59-15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–
59-20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	24	7	–	–	–	–	–
59-22	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–
59-25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
59-26	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
59-28	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
59-29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–
59-38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–
59-50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–
59-51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–
59-52	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	1	15	–	–	1	–	1
59-64	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–
60-1001	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	2	–	–	–
60-1008	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	–	–	–
60-1011	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9	–
60-1012	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–
60-1016	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–
60-1025	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	–
60-1035	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
60-1039	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	24	–
61-1021	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3	2	–	–	2	–	–
61-1026	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	3	–	–	–	69	–
61-1053	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3	1	–	–	–	30	–
61-1062	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	2	–	–	–	–	–	–	–
61-1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	4	–	–	–	–	–
61-2	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	3	2	1	–	–	–	–
61-3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	5	3	–	–	–	–	–
61-4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE IV. (Continued)

TABLE IV. (Continued)

Sta. No.	*Aplidium glabrum*	*Synoicum pulmonaria*	*Didemnum albidum*	Undetermined didemnids	*Polycitor sitkeus*	*Ascidia callosa*	*Ascidia obliqua*	*Ascidia prunum*	*Cheloposoma macleayanum*	*Botryllioides gyrcum*	*Chemidocarpa hmmarkmeis*	*Chemidocarpa rhizopus*	*Dendrodoa aggregata*	*Dendrodoa pulchella*	*Styela cortacea*	*Styela rustica*	*Boltenia ovifera*	*Boltenia echinata*	*Halocynthia pyriformis*	*Molgula griffithsii*	*Molgula alphonalis*	*Rhizomolgula globularis*	Unidentified molgulids
61-5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	5	1	1	1	1	1	1
61-10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	6	1	1	10	1	1	1
61-11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	31	20	1	39	1	1	1
61-12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	6	2	1	1	2	1	1
61-13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
61-15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	3	1	1
61-18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
61-21	1	1	1	1	1	1	1	4	7	1	1	1	4	1	1	1	1	1	1	1	1	1	1
61-24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
61-26	1	4	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1
61-30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1
61-32	1	1	1	1	3	8	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1
61-39	1	1	2	1	1	1	2	1	1	1	1	1	6	1	1	1	1	8	1	1	1	1	1
61-43	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
62-006	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
62-008	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1	1	1	1	1	1	1	1
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62-2031	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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62-3001	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1
62-4001	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
62-4006	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1
62-4009	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1
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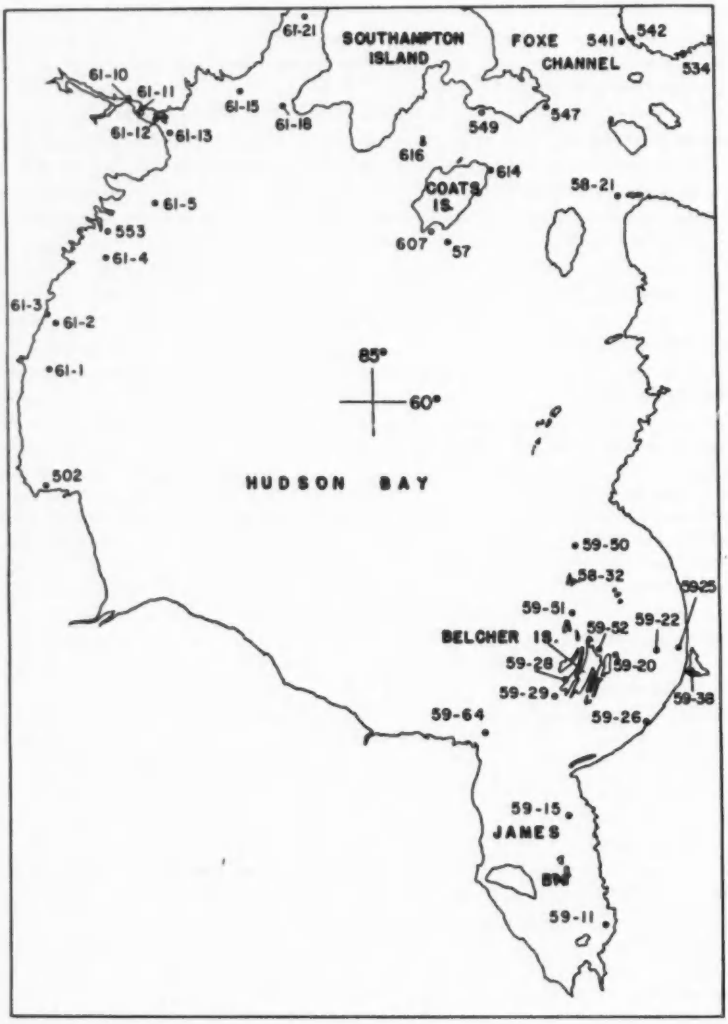
SOUTHAMPTON ISLAND
FOXE CHANNEL
61-21
541
542
534
61-10
61-11
61-15
61-16
549
547
61-12
61-13
616
614
COATS IS.
58-21
61-5
553
607
57
61-4
61-3
61-2
61-1
85°
60°
502
HUDSON BAY
59-50
58-32
5925
59-51
59-52
59-22
BELCHER IS.
59-28
59-20
59-29
59-38
59-64
59-26
59-15
JAMES
59-11

FIG. 2

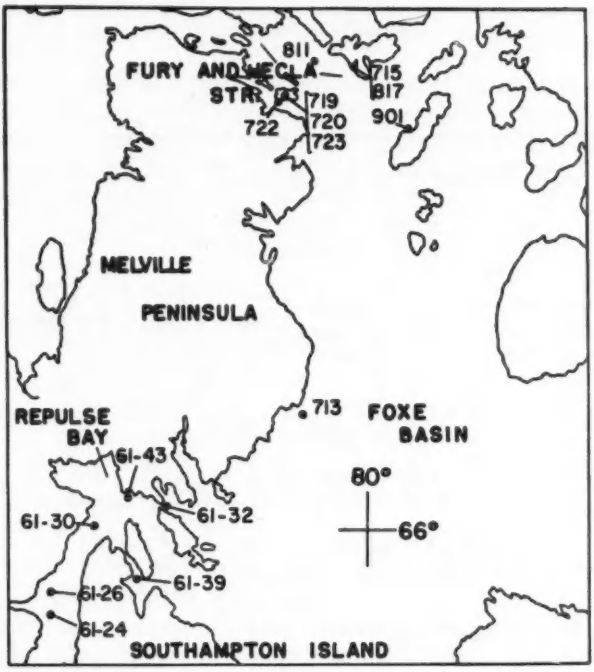
811
FURY AND HECLA STR.
715
817
719
901
722
720
723
MELVILLE
PENINSULA
713
FOXE
BASIN
REPULSE
BAY
61-43
80°
61-32
61-30
66°
61-39
61-26
61-24
SOUTHAMPTON ISLAND

FIG. 3

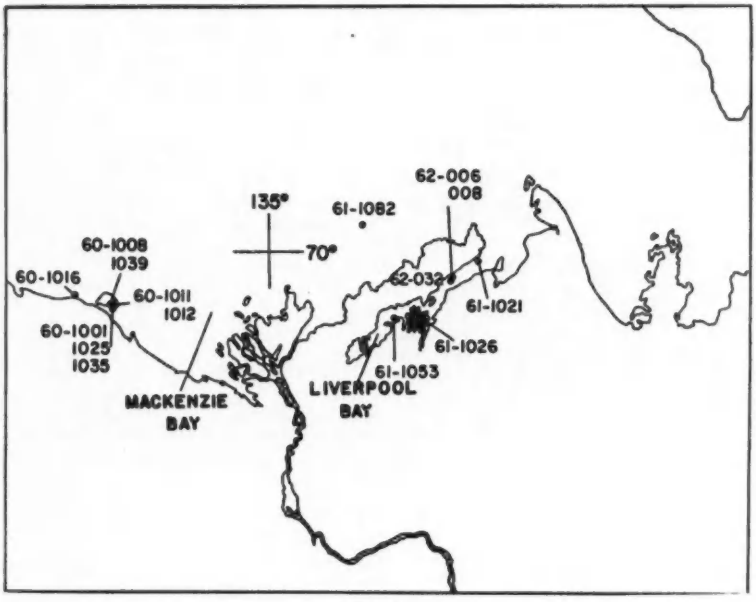
62-006
008
135°
61-1082
70°
60-1008
1039
60-1016
60-1011
1012
62-032
61-1021
60-1001
1025
1035
61-1026
61-1053
LIVERPOOL
BAY
MACKENZIE
BAY

FIG. 4

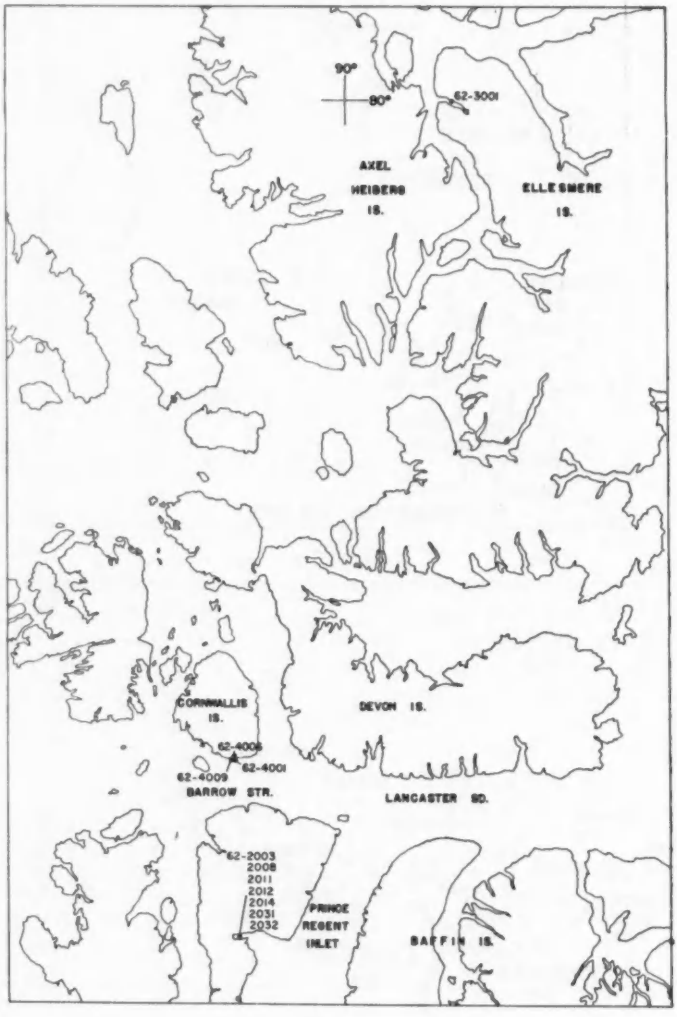
90°
80°
62-3001
AXEL
HEIBERG
IS.
ELLESMERE
IS.
CORNWALLIS
IS.
62-4008
62-4001
62-4009
BARROW STR.
DEVON IS.
LANCASTER SD.
62-2003
2008
2011
2012
2014
2031
2032
PRINCE
REGENT
INLET
BAFFIN IS.

FIG. 5

ACKNOWLEDGMENTS

The author wishes to express her thanks to the Department of Zoology, University of California, Berkeley, and to the Hopkins Marine Station of Stanford University for the use of research space and facilities, and to Dr E. H. Grainger of the Fisheries Research Board of Canada for providing maps with the pertinent station positions and numbers.

REFERENCES

ARNBACK CHRISTIE-LINDE, A. 1922–1934. Tunicata. *In* Northern and Arctic invertebrates in the collection of the Swedish State Museum. *K. Svensk. Vetens. Akad. Handlingar*, Vol. 63, pt. 1 (1922), No. 2, 62 pp.; pt. 2 (1923), No. 9, 25 pp.; ser. 3, Vol. 6, pt. 3 (1928), No. 9, 101 pp.; ser. 3, Vol. 13, pt. 4 (1934).

DUNBAR, M. J., AND E. H. GRAINGER. 1952. Station list of the *Calanus* expeditions, 1947–50. *J. Fish. Res. Bd. Canada*, **9**(2): 65–82.

GRAINGER, E. H. 1954. Station list of the *Calanus* expeditions, 1951–52, together with Frobisher Bay stations, 1948, 1950 and 1951, and Resolution Island stations, 1950. *Ibid.*, **11**(1): 98–105.

GRAINGER, E. H., AND M. J. DUNBAR. 1956. Station list of the *Calanus* expeditions, 1953–54. *Ibid.*, **13**(1): 41–45.

GRAINGER, E. H., AND J. G. HUNTER. 1959. Station list of the 1955–58 field investigations of the Arctic Unit of the Fisheries Research Board of Canada. *Ibid.*, **16**(4): 403–420.

HARTMEYER, R. 1923–24. Ascidiacea. In Danish Ingolf Expedition. Copenhagen, Vol. 2, pt. 6 (1923, 368 pp.), pt. 7 (1924, 278 pp.).

REDIKORZEV, V. 1907. Ein Beitrag zur Ascidienfauna der Arctic. *Ann. Mus. Zool. Acad. St. Petersbourg*, Vol. 11, pp. 126–154.

1907a. Die Ascidien der russischen Polar-Expedition, 1900–03. *Zool. Anz.*, Vol. 31, pp. 521–525.

1910. Ascidians of the Arctic Seas. (In Russian) *Trav. Soc. Nat. St. Petersbourg*, Vol. 41, fasc. 2, pp. 85–175.

VAN NAME, W. 1945. The North and South American ascidians. *Bull. Amer. Mus. Nat. Hist.* Vol. 84, 476 pp.

NOTES

Sterol Metabolism in the Oyster[1]

Marine invertebrates have been shown to be a source of many and varied sterols but the origin of these sterols has not been determined. The nature of the endogenous sterols could depend upon an active biochemical conversion of ingested sterols, or the synthesis of sterols from more basic precursors, or both. Earlier studies demonstrated that starfish can transfer the double bond of cholesterol from the C_5-position to the C_7-position to form 7-cholestenol (Fagerlund and Idler, 1960), and that cholesterol could be converted to 24-methylenecholesterol *in vivo* by the clam (Fagerlund and Idler, 1961). This report describes a preliminary study of the effect of feeding a C_{29}-sterol, β-sitosterol, upon the sterols deposited in the body tissues of the oyster, a species in which C_{27}- and C_{28}-sterols characteristically predominate.

Small oysters, *Crassostrea virginica* (Gmelin), were obtained from the Board's Biological Substation at Ellerslie, P.E.I. The oysters were divided into three groups: group I was killed immediately for the determination of total lipid, unsaponifiable components, sterols and sterol composition; the other two groups, II and III, were transferred to two laboratory holding tanks for the feeding experiments. The tanks were cylindrical glass columns, fitted with perforated platforms to hold the oysters, and equipped with a siphon to allow a one-way flow of water. The holding tanks were supplied with heated running sea water previously filtered through sand and gravel, and then through fine porosity paper (Stewart and Power,.1963) in order to eliminate plankton and to minimize the numbers of bacteria, both food items and possible sources of sterols for the oyster. Under our experimental conditions the water flowing into the holding tanks had to be heated to 20–25°C to promote meat growth.

Two diets were prepared: one was essentially sterol-free, the other was supplemented with β-sitosterol. A concentrated form of the basic diet contained the following ingredients: defatted fish protein, 30%; fat-free starch, 60%; alphacel (Nutritional Biochemicals Corp.), 10%; linoleic acid, 4%; vitamin mixture (per kg diet), vitamin A acetate, 20 mg; vitamin D, 2.5 mg; pyridoxine HCl, 20 mg; thiamine HCl, 20 mg; and folic acid, 2.0 mg. The basic diet mixture was suspended in water and sterilized by cobalt-60 irradiation because conventional methods of sterilization caused the dispersion to gelatinize. The sterol-supplemented diet was prepared by adding 0.5% β-sitosterol to the basic diet. Both diets were diluted with sterilized sea water, and added to the sea water inflow of the oyster tanks by a peristaltic pump at a rate sufficient to give a final concentration of diet in the tanks of 0.0001%. The one-way flow of water through the tanks prevented the accumulation of the diet dispersion in the tanks and inhibited bacterial growth.

[1]This investigation was supported by a research grant from the National Institutes of Health, U.S. Public Health Service.

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The control (Group II) and experimental (Group III) oysters were held under identical conditions except for the diet for a period of 32 weeks. Group II was fed the sterol-free diet and Group III was fed the sterol-supplemented diet. The temperature of the sea water was too low for the first 20 weeks to promote meat growth although the oysters did deposit new layers of shell under these conditions. Sampling of the oysters was carried out after holding for 20 weeks, and 32 weeks. Before the oysters were removed from the tanks, feeding was stopped and the seawater flow was continued in order to remove undigested sterols from the oysters. The sampled oysters were removed from the shell and the meat was analyzed for total lipids, unsaponifiable components, and sterols. The results are listed in Table I.

TABLE I. Per cent composition of the meat of oysters before and after live holding.

Group	Holding time (*weeks*)	Weight of meat[d]	Total lipid	Unsaponifiable components	Sterols
		g	%	%	%
I	0	58	2.0	0.41	0.14
II[a]	20[c]	10	0.9	0.19	0.054
III[b]	20	16	0.9	0.49	0.10
II	32	11	0.7	0.22	0.077
III	32	12	1.0	0.27	0.12

[a]Oysters maintained on sterol-free diet.
[b]Oysters maintained on β-sitosterol-supplemented diet.
[c]Temperature of the water in the holding tanks was too low (below 20°C) to promote growth during the first 20 weeks.
[d]Each oyster contained approximately 2 g of meat.

The composition of the sterols of the oysters, before and after holding on artificial diets, was determined by gas chromatography. The apparatus used was a Barber-Colman Model 10 with an argon ionization detector and glass columns (4 mm ID, 6 ft in length). Peak areas on the chromatogram were measured by a Disc Co. integrator and assumed to be equal to weight per cent. The results of the chromatographic analyses of the total sterols are given in Table II. In order to determine any change in the β-sitosterol content of the sterols, the methyl ether derivatives were prepared (Clayton, 1962) and chromatographed (Table III). The peak areas of the chromatograms were identified by comparison of column retention times (r_c), relative to cholestane, with the retention times of authentic compounds and of scallop sterols (Tamura *et al.*, 1964; Wainai *et al.*, 1964).

The results of this experiment indicate that a sterol-free diet does not inhibit the growth of the oyster, at least for 3 months. However, the depletion of sterols in the flesh of the oysters on a sterol-free diet (Table I) suggests that oyster sterols are at least in part of exogenous origin. Certainly, the β-sitosterol (peak area M_7, Table III) added to the diet of the experimental group

TABLE II. Composition of the sterols of oysters as determined by gas chromatography on a column of 5% SE-30 on Anakrom ABS 70/80 mesh; argon pressure of 30 psi and temperatures (°C) were: column, 230; flash heater, 310; detector cell, 275.

Weight % of peak areas[a]

(r_c = column retention time relative to cholestane)

Oysters group	Holding time (*weeks*)	X r_c: 1.28	A 1.83	B 2.03	C 2.30	D 2.66	E 3.5
I	0	4	8	40	27	11	10
II[b]	20[d]	5	9	44	26	9	7
III[c]	20	5	8	45	25	10	7
II	32	5	6	55	21	9	4
III	32	4	5	45	15	11	20

[a]The main components of sterols represented by peak areas of the chromatogram were determined previously as follows: X, C_{26}-compounds; A, 22-dehydrocholesterol; B, cholesterol; C, brassicasterol or its C-24 stereoisomer; D, 24-methylenecholesterol; E, C_{29}-sterols (Wainai *et al.*, 1964).

[b]Oysters maintained on sterol-free diet.

[c]Oysters maintained on β-sitosterol-supplemented diet.

[d]Temperature of the water in the tank was too low (below 20°C) to promote growth during the first 20 weeks.

TABLE III. Composition of the methyl ether derivatives of oyster sterols as determined by gas chromatography on a column of 5% diethyleneglycol succinate (DEGS) on Chromosorb W; argon pressure, 15 psi and temperatures (°C) were: column, 195; flash heater, 350; detector cell, 212.

Weight % of peak areas[a]

(r_c = column retention time relative to cholestane)

Oysters group	Holding time (*weeks*)	M_1 r_c: 2.3	M_2 3.2	M_3 3.66	M_4 4.4	M_5 5.6	M_6 5.6	M_7 6.2	M_8 7.1	M_9 7.4	M_{10} 8.2
I	0	3.6	0.5	43.7	23.8	2.8	8.8	4.2	1.6	8.9	1.9
II[b]	20[d]	3.3	1.0	51.6	22.7	2.7	7.9	4.9	0.7	3.8	1.4
III[c]	20	4.8	1.3	47.0	22.3	3.5	9.5	4.1	0.7	5.9	0.9
II	32	4.1	0.9	56.8	19.8	3.0	7.8	4.2	1.4	1.4	0.9
III	32	3.8	0.8	45.0	14.0	5.4	7.3	19.8	3.9		–

[a]Some of the methyl ether derivatives of sterols represented by peak areas of the chromatogram have been identified as follows: M_2, C_{27} saturated sterol; M_3, 22-dehydrocholesterol and cholesterol; M_4, brassicasterol; M_5, C_{28} Δ^5-sterol; M_6, 24-methylenecholesterol; M_7, β-sitosterol; M_8, fucosterol; M_9, C_{29} unsaturated sterol, possibly 20-isofucosterol.

[b]Oysters maintained on sterol-free diet.

[c]Oysters maintained on β-sitosterol-supplemented diet.

[d]Temperature of water in holding tanks was too low (below 20°C) to promote growth during the first 20 weeks.

of oysters was digested by the oyster and accumulated in the endogenous sterols. The relative cholesterol content (peak area C, Table II) increased in the flesh of the oysters on a sterol-free diet but remained relatively constant for the experimental animals. In both groups, the relative 24-methylenecholesterol (peak area D, Table II) content remained constant. The oysters apparently were capable of synthesizing cholesterol and conceivably could maintain 24-methylenecholesterol levels by conversion of cholesterol as shown in an earlier study (Fagerlund and Idler, 1961). The persistent decrease in the amounts of a C_{29}-sterol, tentatively identified as 20-isofucosterol (peak area M_9, Table III) in both groups of oysters would indicate that this compound may be an essential sterol for the oyster. Further studies using radioactive sterols could further clarify the results of this investigation and explain the effects of sterols in the diet on the endogenous sterols of the oyster.

The authors thank Mr R. Drinnan of the Board's Biological Substation, Ellerslie, P.E.I., for supplying the live oysters and for his advice concerning the maintenance of the oysters in the laboratory. Thanks are also due to Dr R. G. Ackman for assistance with the gas chromatography and to Mrs L. Buyko for technical assistance.

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REFERENCES

CLAYTON, R. B. 1962. Gas liquid chromatography of sterol methyl ethers and some correlations between molecular structure and retention data. *Biochemistry*, **1**: 357–366.

FAGERLUND, U. H. M., AND D. R. IDLER. 1960. Marine sterols. VI. Sterol biosynthesis in molluscs and echinoderms. *Canadian J. Biochem. Physiol.*, **38**: 997–1002.

1961. Marine sterols. IX. Biosynthesis of 24-methylenecholesterol in clams. *Ibid.*, **39**: 1347–1355.

STEWART, J. E., AND H. E. POWER. 1963. A sea water aquarium for marine animal experiments. *J. Fish. Res. Bd. Canada*, **20**: 1081–1084.

TAMURA, T., T. WAINAI, B. TRUSCOTT AND D. R. IDLER. 1964. Isolation of 22-dehydrocholesterol from scallop. *Canadian J. Chem.*, **42**: 1331–1337.

WAINAI, T., T. TAMURA, B. TRUSCOTT AND D. R. IDLER. 1964. The application of gas chromatography to the identification of the sterols of scallop. *J. Fish. Res. Bd. Canada*, **21**: 1543–1546.

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Quest for the True Breeding Area of the American Eel (*Anguilla rostrata* LeSueur)

After 20 years of exhaustive studies, Johannes Schmidt published in 1922 and then further in 1925 his findings on the life history of the European eel (*Anguilla anguilla* Linnaeus). In these publications he outlined the breeding areas for both the European eel (*A. anguilla*) and the American eel (*A. rostrata*). Apparently his conclusions satisfied the needs of the scientific world for many years (Bertin, 1951, 1956).

Three decades later, Tucker (1959a) published a startling article: "A new Solution to the Atlantic Eel Problem". He claimed that the American and European eels were not distinct species, but merely eco-phenotypes of *A. anguilla*, their distinguishing characters being determined by differences in the temperatures of the spawning areas. Moreover, he felt that the populations of the so-called "European" eels were entirely maintained by reinforcements of larvae of "American" parentage.

This ingenious hypothesis formed the basis of Tucker's "New Solution" which, however, did not meet with the approval of European authors, and several criticisms were published. The following critics should be mentioned: D'Ancona (1959), Deelder (1960), Jones (1959), and Bruun (1963) who has offered the most substantiated objection. Tucker, in reply to his critics, published three subsequent articles (1959b, c, 1960). The value of Tucker's publications lay not in his "New Solution" to the Atlantic eel problem, which he did not resolve, but in the fact that he showed clearly that many of Schmidt's assumptions about spawning areas were based on circumstantial evidence rather than on proven facts. This was particularly true in the case of the American eel.

ANALYSIS OF JOHANNES SCHMIDT'S DATA

Although Schmidt's classical studies pointed out definitely that the spawning areas of the European and American eel species were located in the southwest part of the North Atlantic, the known facts seem insufficient to claim that the breeding place for the American eel (*A. rostrata*) has been precisely established. Several strong objections to Schmidt's claim can be raised.

1. *Lack of knowledge on the degree of maturity of eels during their seaward migration.* The only information available is restricted to the exterior appearance of sea-going European eels referred to as "silver eels" (Petersen, 1896:8–17) and to sea-going female eels in North America (Quebec) as "bronze eels" (Vladykov, 1955). In neither case were the conditions of the gonads of sea-migrating eels sufficiently studied.

Boetus *et al.* (1962), and Bruun (1963:149), observed that the egg diameters were from 0.20 to 0.35 mm in four specimens of *A. rostrata* sent by me to Copenhagen in 1959. These female American eels, caught in the St. Lawrence River near Quebec City, were large fish, weighing on the

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average 1280 g (range: 920–1835 g). The maturity stage of the Canadian fish was compared with the much smaller *A. anguilla* from Denmark, which weighed on the average 715 g (range: 510–1075 g). The diameters of the eggs in the Danish fish were smaller than those from Canada. This difference could be attributed either to different degrees of maturity or to the size of the fish or to both.

In Europe, *A. anguilla* in advanced stages of maturity, with their remarkably large eyes (macrophthalmia stage), have been captured several times. This was reported by Schmidt (see Bertin, 1956:105), Svardson (1949), Rasmussen (1952), to mention a few. In America, so far, no eels with remarkably large eyes have been described.

2. *Lack of spawning eels and their shed eggs taken in the sea.* Schmidt established the breeding areas for both Atlantic species not by capturing spawning eels over what was presumably their breeding place nor by collecting their eggs[1], but merely by catching relatively young larvae. Numerous 7–15-mm leptocephali of *A. anguilla* were obtained by Schmidt but only the odd larvae, 7–8 mm long, belonged to *A. rostrata*. Since the ages of these were unknown, the larvae could have originated in places other than those where they were caught. Schmidt (1925) placed the spawning area of the European eel between latitudes 22° and 30° N and between longitudes 48° and 65° W, with its central part nearly equidistant from Puerto Rico and Bermuda (Bertin, 1956:120). Schmidt (1925:310) centred the breeding place of *A. rostrata* somewhat further west and south (Fig. 1).

[1]The claim by Fish (1927) that she succeeded in hatching true eggs of *A. rostrata* taken near Bermuda is discounted by Danish authors (Tåning, 1938; Bruun, 1963).

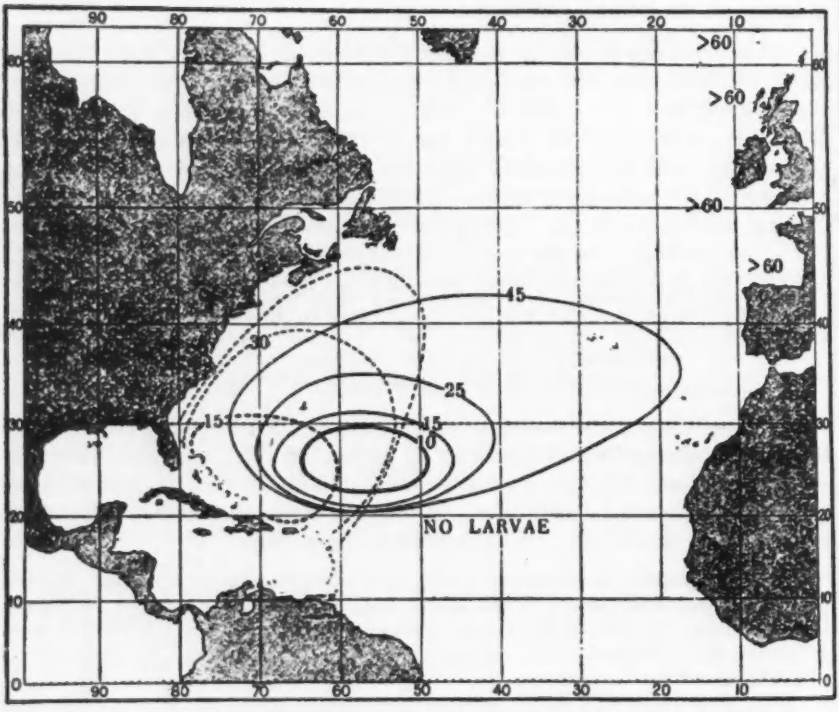

FIG. 1. Presumed breeding areas for the European eel (*Anguilla anguilla*) and the American eel (*A. rostrata*). (After Schmidt, 1925.) Continuous curves refer to the European eel, and dotted ones to the American eel. The numbers 10,15, etc., indicate the maximum length in millimetres of leptocephali found within the respective areas.

3. *Inadequate distribution of the Danish hydrographical stations.* The principal purpose of Schmidt's studies was to locate the birthplace of the European eel[2]. As Tucker (1959a) put it, "the *A. rostrata* larvae[3] were to him an unexpected nuisance". Thus the Danish Expeditions made numerous hauls at places where the leptocephali of *A. anguilla*, and not *A. rostrata*, were most abundantly found. An examination of Fig. 2 revealed that the Danish stations, 1903–22, were confined to the area between latitudes 20–30° N and longitudes 50–70°W. Very few stations were established between latitudes 10–20°N, and only odd hauls were taken further south by the "Dana II" Expedition, 1921–22 (Schmidt, 1935).

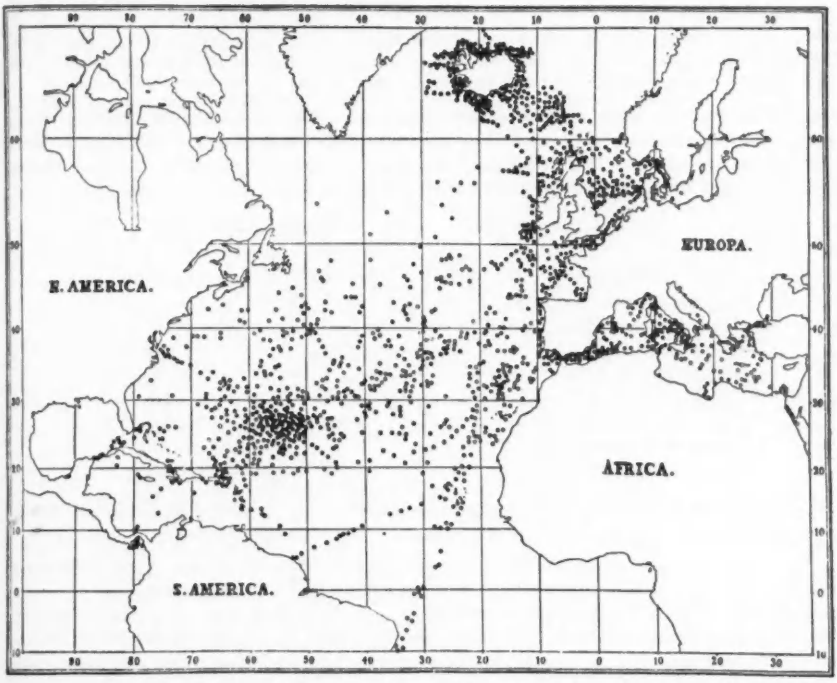

FIG. 2. Principal Danish collecting stations, 1903–1922. (After Schmidt, 1925.) Closed circles indicate stations by research ships and open circles those by other ships.

4. *Limited bathypelagic distribution of samples.* On several occasions Schmidt (1925:305–306) found that leptocephali of *A. anguilla* and *A. rostrata* were taken together. Nevertheless, each species apparently had its own bathypelagic preference. Tåning (1938) reported that in 1350 hauls made off Nonsuch Island by the Bermuda Oceanographic Expeditions, from April to November during 1929–31, only 46 leptocephali of both species were collected and they were taken at the following depths:

Depth (*fathoms*)	*Total no. of hauls*	*A. anguilla* (18–50 *mm*)	*A. rostrata* (39–53 *mm*)
25–200	63	12	–
300–1000	1112	17	17

[2]The larvae of the European eel were described by Kaup (1856) under the name of *Leptocephalus breviceps*.

[3]The larvae of the American eel were named *Leptocephalus grassii* by Eigenmann and Kennedy (1902).

This very limited number of leptocephali does not necessarily indicate their scarcity in the western North Atlantic, but may reflect simply that either the stations selected were in an area little frequented by leptocephali, or that the collections were made during an inappropriate season, or at the wrong depths. It should be noted that Schmidt (1925: 297–98) reported occasional extensive catches of leptocephali of either species. In a 2-hr haul at about 50 m depth, station 871 (lat 27°15′ N, long 61°35′ W), on June 27, 1920, almost 800 leptocephali of *A. anguilla* were taken.

The best catches of leptocephali of *A. rostrata* were made at "Dana" station 948 (lat 22°14′N, long 67°22′W), on April 27, 1921, when 229 leptocephali were taken, and at "Dana" station 891 (lat 29°28′N, long 69°25′W), on July 24, 1920, when 219 larvae were obtained (Schmidt, 1925: 308). He concluded that the larvae of the two species were by no means evenly distributed throughout the area. West of about 62°W long and south of about 24°N lat, *A rostrata* predominated over *A. anguilla*.

5. *Small numbers of* A. rostrata *leptocephali in collections.* Schmidt (1925) was well aware that in the hauls made by the Danish Expeditions the leptocephali of both Atlantic species were not equally represented. The following table, based on data by Schmidt (1925) and Tåning (1938), suggests the wide variation in abundance of leptocephali of the two species:

	Number of leptocephali	
Collecting data	*A. rostrata*	*A. anguilla*
Captain Andrea (about 1865)	1	2
Trading vessels, 1911–15	3	120
Margrethe, 1913	24	714
U.S.S. *Bache*, 1914	6	31
Dana, 1920–21	1868	6446
Dana II, 1921–22	>400	>4500
Bermuda Oceanographic Expedition, 1929–31	17	29

Commenting on such inequality, Schmidt (1925: 307) said: "In all the collections the European eel predominates, even in the western Atlantic. This is possibly the case also in reality."

6. *Lack of knowledge on the precise season of spawning of both species of eels.* The catadromous migration of silver eels in Europe takes place in the autumn. The principal fishery of the American eel also coincides with the autumn months, from the end of August to the end of November. However, there is some information (Smith and Saunders, 1955) that in certain small New Brunswick lakes, such as Crecy and Gibson, small eel runs occur in April and May and larger runs take place from late August to November. I found that among the adult eels from Crecy Lake, males predominate, representing the highest proportion of males in Canadian samples studied. Does this signify that in North America the male eels depart seaward earlier than females?

On the basis of Schmidt's studies, Bertin (1956: 169) concluded that "another biological peculiarity of the American eel is its earlier spawning, which begins in January to February, not in February to March as with the other species".

It should be noted that I was able to keep two bronze female eels from the St. Lawrence River without food for 22 months before they died. Professor U. D'Ancona (personal communication) succeeded in keeping European silver eels for an even longer period without food. Thus we must not forget that both Atlantic species of eels, prior to their seaward migration, have firm and fat bodies, a condition which helps them to sustain a long exhaustive migration towards their respective spawning areas. Therefore, the breeding ground of the American eel must be situated as far from the American shore as is that of the European eel, explaining the excellent physical condition of the migrating American eels.

7. *Limited information on the duration of the larval life of* A. rostrata *and its migration towards the coast.* The most striking discovery by Schmidt (1922) was that the leptocephali of both species did not grow at the same rate. According to him the American leptocephali hatched in

February and at the end of the year their maximum size was 60–65 mm. In winter they metamorphosed into elvers which invaded the continental waters the following spring. As Bertin (1956: 169) put it: "the same series of phenomena takes three years in the European eel, so that the American species develops in a third of the time and must therefore complete its migration three times as quickly". It is the duration of the larval life which sorts out the European and the American larvae. Those which metamorphose earlier populate America; those later, populate Europe[4].

Although the complex surface currents of the Gulf Stream[5] are apparently essential for bringing leptocephali of *A. anguilla* back to Europe, they must interfere greatly with the normal distribution of the American eel larvae. To reach North America, *A. rostrata* leptocephali must somehow cross the Gulf Stream without being swept away to the northeast Atlantic (Fig. 3).

[4]Bruun (1937 : 26) reported that a single *A. rostrata* has been found in northern Spain; it was found among several hundred *A. anguilla* whose vertebrae were counted.

[5]For details on the circulation in the western North Atlantic along the Canadian Coast, see Hachey (1961).

FIG. 3. Surface currents of the Atlantic Ocean in February–March. (After Sverdrup *et al.*, 1959.) The large black arrows indicate the direction of the North Equatorial Current.

8. *Lack of explanation of southernmost distribution of* A. rostrata. Schmidt (1925: 304 and 307) gave the following north–south limits for the eel larvae:

Anguilla rostrata	*Anguilla anguilla*
Northenmost find: Lat 42°17′N, long 50°22′W	Lat 42°19′N, long 50°22′W
Southernmost find: Lat 17°55′N, long 64°48′W	Lat 20°14′N, long 57°03′W

According to Bertin (1956: 170) and Jensen (1937), adult *A. anguilla* extend from 23° to 70°N lat and adult *A. rostrata* from 5° to 62°18′N lat.

The geographical distribution of the larvae and adults of *A. rostrata* is very extensive, from southwestern Greenland to the Guianas. A northern branch of the Gulf Stream (Irminger Current) could help the leptocephali to breach the open waters between Newfoundland and Greenland (Jensen, 1937).

It is much more difficult to explain the presence of adult *A. rostrata* over their southernmost limits. How can they reach Trinidad[6] and the Guianas[7] which are located about 13° south from the supposed breeding area in the Sargasso Sea? The strong North Equatorial Current passes along the coast of South America, directing north (Sverdrup *et al.*, 1959), thus making migration of leptocephali from the Sargasso Sea to the south apparently impossible.

CONCLUSION

In all fairness to the late Johannes Schmidt it should be said that his classical studies on different species of eels (*Anguilla*) are extremely helpful to understand the essential points of their biology. His findings on the duration of the larval stages, distribution, and ways of migration of *A. anguilla* leptocephali do not raise any objection. The outline of the presumed spawning area for *A. anguilla* seems most reasonable as well.

However, additional and more complete studies of the distribution of leptocephali and elvers of *A. rostrata* and particularly of the location of their spawning areas are needed. How can one explain the inequality of specimens obtained: about 2300 leptocephali of *A. rostrata* compared to about 12,000 of *A. anguilla* taken by Schmidt during the whole period of 1911–1921? How can leptocephali of the American eel reach Trinidad and the Guianas against the strong North Equatorial Current, if they are hatched in the Sargasso Sea? It was well established by Schmidt that the leptocephali cannot migrate actively to any extent but are passively carried by the currents.

The only reasonable answer to the above questions is in the assumption that the *true spawning place for* A. rostrata *is not located in the area outlined by Schmidt (1922) but much further south.*

There are several questions which still await answers:

1. Where in the western North Atlantic is the true spawning area of *Anguilla rostrata*?

2. What are the conditions of the spawning area, such as the depths and temperatures?

[6]From Trinidad (Shark River) I was able to examine three samples of adult *A. rostrata*, collected on the following dates: August 21, 1959 (9 fish), January 8, 1960 (8 fish), and December 1963 (21 fish).

[7]Schmidt (1909: 13) reported the presence of adult *A. rostrata* in both British Guiana and Dutch Guiana.

3. Do the males and females of the American eel undertake seaward migration at different seasons?

4. At what depths and at what temperatures do the American eels swim in the sea during the spawning migration?

5. Do they always follow the same route?

6. How do the leptocephali and elvers of *A. rostrata* avoid the Gulf Stream in order to reach the North American continent?

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REFERENCES

BERTIN, L. 1951. Les anguilles. Payot, Paris. 191 pp.

1956. Eels. A biological study. Cleaver-Hume Press, London VIII, 192 pp.

BOETIUS, J., I. BOETIUS, A. M. HEMMINGSEN, A. FR. BRUUN, AND E. MØLLER-CHRISTENSEN. 1962. Studies of ovarial growth induced by hormone injections in the European and American eel (*Anguilla anguilla* L. and *Anguilla rostrata* Le Sueur). *Medd. Danmarks Fisk. Havundersøg.*, N.S., **3**(7): 183–198.

BRUUN, A. FR. 1937. Contributions to the life histories of the deep sea eels: Synaphobranchidae. *Dana Rep.*, **9**: 1–39.

1963. The breeding of the North Atlantic fresh-water-eels. *Advances Mar. Biol.*, **1**: 137–169.

D'ANCONA, U. 1959. Old and new solutions to the eel problem. *Nature*, **183**: 1405. London.

DEELDER, C. L. 1960. The Atlantic eel problem. *Nature*, **185**: 589–591. London.

EIGENMANN, C. H., AND C. H. KENNEDY. 1902. The leptocephalus of the American eel and other leptocephali. *U.S. Fish Comm. Bull.*, **21**: 81–92.

FISH, M. P. 1927. Contributions to the embryology of American eel. *Zoologica*, **8**: 289–324.

HACHEY, H. B. 1961. Oceanography and Canadian Atlantic waters. *Bull. Fish. Res. Bd. Canada*, No. 134, 120 pp.

JENSEN, AD. S. 1937. Remarks on the Greenland eel, its occurrence and reference to *Anguilla rostrata*. *Medd. Grønland*, **118**(9): 1–8.

JONES, J. W. 1959. Eel migration. *Nature*, **184**: 1281. London.

KAUP, J. J. 1856. Catalogue of apodal fish in the collection of the British Museum, London.

PETERSEN, C. G. J. 1896. I. The common eel (*Anguilla vulgaris*, Turton) gets a particular breeding-dress before its emigration to the sea. The bearings of this fact on the classification and on the practical eel-fisheries. *Rep. Danish Biol. Sta.*, **5**: 5–37. Copenhagen, 1894–1895.

RASMUSSEN, C. J. 1951. Two Danish finds of female eel (*Anguilla anguilla*) in spawning or partial spawning dress. *Rep. Danish Biol. Sta.*, **53**: 35–39.

SCHMIDT, J. 1909. On the distribution of the fresh-water eels (*Anguilla*) throughout the world. I. Atlantic Ocean and adjacent regions. A biogeographical investigation. *Medd. Komm. Havundersøg.*, **3**(7): 45 pp.

1922. The breeding places of the eel. *Phil. Trans., Series B.*, **211**: 179–208.

1925. The breeding places of the eel. Smithsonian Report for 1924, pp. 279–316.

1935. Danish eel investigations during 25 years, 1905–1930. Edited by Carlsberg Foundation. 16 pp. Copenhagen.

SMITH, M. W., AND J. W. SAUNDERS. 1955. The American eel in certain fresh waters of the Maritime Provinces of Canada. *J. Fish. Res. Bd. Canada*, **12**(2): 238–269.

SVÄRDSON, G. 1949. Eels (*Anguilla anguilla*) found in Sweden in partial nuptial dress. *Rep. Inst. Freshwater Research, Drottningholm*, **29**: 129–134.

SVERDRUP, H. U., M. W. JOHNSON, AND R. H. FLEMING. 1942. The oceans. Prentice-Hall, Inc., New York, 1087 pp.

TÅNING, A. V. 1938. Deep-sea fishes of the Bermuda Oceanographic Expeditions. Family Anguillidae. *Zoological*, **23**(3): 313–318.

TUCKER, D. W. 1959a. A new solution to the Atlantic eel problem. *Nature*, **183**: 495–501. London.

1959b. (Answering D'Ancona, 1959). *Ibid.*, **183**: 1406.

1959c. (Answering Jones, 1959). *Ibid.*, **184**: 1281–1283.

1960. (Answering Deelder, 1960). *Ibid.*, **185**: 591–592.

VLADYKOV, V. D. 1955. Eel, fishes of Quebec. Album No. 6, pp. 1–12. Dept. of Fisheries Quebec.

Demersal Quality of Fertilized Eggs of Rock Sole (*Lepidopsetta bilineata* Ayres)

Smith (1936) reported that *Lepidopsetta bilineata* in Puget Sound, Washington, produces buoyant non-adhesive eggs. On the other hand, Pertseva-Ostroumova (1961) reported that in the northwestern Pacific the eggs of that species are demersal and adhesive. Such differences are not entirely unexpected in view of Moiseev's (1955) comments on modifications in reproduction of some far-eastern (northwestern Pacific) fish. However, tests conducted at this laboratory support the conclusion that the eggs are demersal and adhesive.

In 1961 and 1962, rearing experiments were conducted on eggs obtained from rock sole in the Strait of Georgia. It was noted that fertilized eggs placed in a container of sea water sank to the bottom and were adhesive. More recently, experiments were conducted to determine the specific gravity of the rock sole egg.

Ripe specimens of male and female rock sole were obtained in Ganges Harbour, Strait of Georgia, and eggs and milt were stripped into a bucket of sea water. A subsample of these eggs (subsequently found to be fertilized and viable) was placed in a glass container of sea water to which common table salt was added until the egg became suspended in the solution. Titration of the solution yielded a salinity of 65‰ from which a calculated specific gravity of 1.048 at 7°C was obtained. The experiment was repeated with specimens taken at another location in the Strait of Georgia (at Neck Point near Nanaimo). The specific gravity of the solution as measured by an hydrometer was found to be 1.047, again at 7°C.

The maximum salinity of sea water overlying the continental shelf adjacent to British Columbia is about 34‰ (Barber and Tabata, 1954). Specific gravity of such water at 7°C would be about 1.027, a value much lower than that of the solution which suspended the rock sole eggs. From this, one would conclude that the eggs of the rock sole would sink to the bottom.

These observations are difficult to reconcile with Smith's findings, particularly in view of the fact that Ganges Harbour and Neck Point are only about 70 and 90 miles, respectively, from Puget Sound.

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REFERENCES

BARBER, F. G., AND S. TABATA. 1954. The Hecate oceanographic project. *Fish. Res. Bd. Canada, Pacific Prog. Rept.*, No. 101, pp. 20–22.

MOISEEV, P. A. 1955. Influence of oceanologic regimen of the far-eastern seas on commercial fish population. Proceedings of the UNESCO Symposium on Physical Oceanography, pp. 253–259.

PERTSEVA-OSTROUMOVA, T. A. 1961. [Reproduction and development of far-eastern flounders.] Razmnokhenie i razvitie dalnevostochnykh kambal. Akademiia Nauk SSSR, Institut Okeanologii, Moscow 1961, 486 pp. [Partial translation by W. E. Ricker.]

SMITH, RICHARD T. 1936. Report on the Puget Sound otter trawl investigations. *State of Washington Dept. Fisheries, Biological Report* No. 36B, 61 pp.

Rate of Development of Eggs of Rock Sole (*Lepidopsetta bilineata* Ayres)

On February 21, 1962, eggs were obtained from ripe rock sole (*Lepidopsetta bilineata* Ayres) taken with a trawl net from a depth of 9–11 fathoms off Neck Point, Strait of Georgia (49°15′ N lat, 124°0′ W long). Fertilization was effected by stripping eggs and milt into a plastic container of sea water and gently stirring the mixture. Within 1 hr of fertilization the eggs were transferred to the laboratory and placed in four wide-mouth Erlenmeyer flasks (500 ml) containing sea water with salinity of approximately 27‰. The flasks in turn were placed in four constant temperature baths (5, 7, 9, and 11°C) maintained within ±0.1°C by thermoregulators. Water in the flasks remained unchanged during the course of the experiment, but aerators were used in each.

The eggs (average diameter 0.92 mm, range 0.87–1.00 mm) were observed to be demersal and adhesive (see Forrester, 1964). Development was not

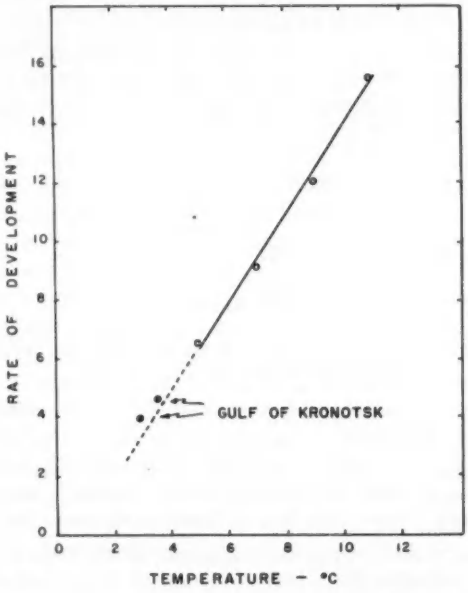

FIG. 1. Rate of development to hatching time for rock sole eggs from the Strait of Georgia, British Columbia (open circles) and from southern Kamchatka (closed circles). The ordinate scale is 100 times the reciprocal of the number of days to first hatching.

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observed at sufficiently frequent intervals to permit documentation of embryonic growth or to determine accurately the time when 50% of the larvae had hatched, but the time of observation of first larva in each flask was recorded. The time to first hatching (considered to be half way between the time of the observation when larvae were first observed and the time of the immediately preceding observation) was found to be 6.4, 8.3, 10.9, and 15.3 days, respectively, for temperatures of 11, 9, 7, and 5°C. From these data a "velocity curve" has been calculated following the method of Krogh (1914). Using Krogh's notation of V as the velocity or rate of development (expressed as 100 times the reciprocal of t, the time in days from fertilization to first hatching), and T as the temperature in °C, the data have the relationship

$$V = 1.506T - 1.202,$$

which is shown in Fig. 1. The relationship is close to linear.

Pertseva-Ostroumova (1961) indicates that time to first hatching of rock sole eggs from the Gulf of Kronotsk (southern Kamchatka) was 25 days and 21.6 days at temperatures of 2.9 and 3.5°C, respectively. The rates of development, as shown in the figure, are close to the expected values obtained by extrapolation of the curve for Strait of Georgia fish.

In the Strait of Georgia, water overlying the spawning grounds during the months of February and March ranges from about 6.5 to 8.0°C (Waldichuk, 1957). At these temperatures rock sole larvae would commence emerging 9–12 days after fertilization.

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REFERENCES

FORRESTER, C. R. 1964. Demersal quality of the fertilized eggs of rock sole (*Lepidopsetta bilineata* Ayres). *J. Fish. Res. Bd. Canada*, **21**(6): 1531–1532.

KROGH, A. 1914. On the influence of temperature on the rate of embryological development. *Z. Allgem. Physiol.*, **16**: 163–177.

PERTSEVA-OSTROUMOVA, T. A. 1961. [Reproduction and development of far-eastern flounders.] Razmnokhenie i razvitie dalnevostochnykh kambal. Akademiia Nauk SSSR, Institut Okeanologii, Moscow 1961, 486 pp. [Partial translation by W. E. Ricker.]

WALDICHUK, MICHAEL. 1957. Physical oceanography of the Strait of Georgia. *J. Fish. Res. Bd. Canada*, **14**(3): 321–486.

On Passive Features in the Osmoregulatory Adaptation of Anadromous Salmonids to Sea Water

The natural movement or experimental transfer of juvenile anadromous salmonids from fresh water into sea water is followed by transient, but marked disturbances of water–electrolyte balance (Black, 1951; Gordon, 1959; Houston, 1959, 1960; Parry, 1960, 1961). Recent studies upon the mechanisms used by these animals to effect osmoregulatory adaptation to marine conditions have, in general, emphasized the importance of changes in extrarenal salt excretion and kidney function, since the establishment of regulated conditions within the body fluid system of the marine teleost depends ultimately upon salt handling and water conservation processes (Holmes and Stott, 1960; Holmes and McBean, 1963; Threadgold and Houston, 1964). It would seem pertinent at this time, however, to emphasize that evidence has accumulated which points to the importance of the effects of another group of processes during the *initial* stages of adaptation. These processes, which include redistribution of tissue water, uptake of inorganic ions by soft tissues and bone, and variations in the permeability of surface tissues, act, as it were, as a kind of "osmoregulatory buffer system" restricting the rate and magnitude of changes in body fluid electrolyte composition. Since they appear to be passive, in the sense of requiring little or no direct energy expenditure, they provide at slight metabolic cost to the animals an interval during which the specialized energy-consuming regulatory activities which eventually restore the regulated state may develop.

Evidence relating to the occurrence of tissue fluid shifts during osmoregulatory adaptation to sea water is to some extent conflicting. Gordon (1959), for example, observed no change in the "chloride space" of the brown trout (*Salmo trutta*), although muscle water content declined following entry into sea water. Parry (1961), and Houston (1959), on the other hand, have interpreted changes in tissue water and potassium concentrations in the Atlantic salmon (*S. salar*), and "chloride space" in the steelhead trout (*S. gairdneri*) as indicative that a transient shift of cellular fluids into the extracellular compartment took place. In the latter species the "chloride space" was observed to increase from a mean of close to 150 g/kg to a maximum of about 225 g/kg, while the volume of the cellular phase dropped from approximately 650 g/kg to a minimum of 570 g/kg. In fish completely adapted to sea water the volume of the cellular phase was close to that seen in freshwater-adapted fish, while extracellular phase volume levelled off at values some 10% greater than those characteristic of premigrant juveniles.

The basis of such shifts presumably lies in the exclusion of sodium and chloride from the cell fluids. The uptake of these ions following entry of the fish into sea water must, then, lead to a condition of hypertonicity of the extracellular fluids relative to those in the cells. Since the two phases are believed to be in osmotic equilibrium this must lead in turn to spontaneous exosmosis of

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cellular fluids. In the steelhead trout the effect of this is seen as an increase of approximately 45% in extracellular phase volume, and a corresponding decrease of about 10% in cellular phase volume.

The value of passive shifts of fluid in the dilution of incoming extracellular electrolytes at the cost of a relatively small degree of cellular dehydration would seem to be qualitatively obvious. Quantitatively the magnitude of this effect is difficult to determine. One may, however, make a rough estimate by calculating the changes in extracellular electrolyte concentration which would occur if the salt load taken up by the animals was confined to an extracellular phase comparable in volume to that characteristic of the freshwater-adapted juvenile stages. Such a calculation, carried out for chloride ion in the steelhead trout (Fig. 1), may be regarded as at least suggestive of the value of passive redistributions of tissue fluids in the restriction of extracellular electrolyte concentration increases during the adaptation process.

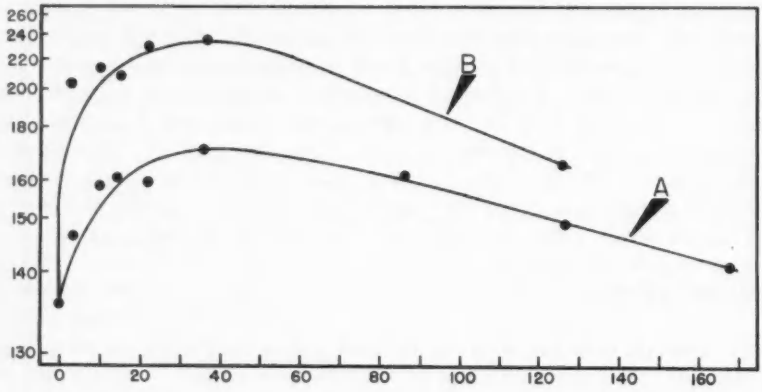

FIG. 1. A. Observed variations in plasma chloride. B. Variations in extracellular chloride calculated upon the assumption of no change in "chloride space". Abscissa—hours in sea water (22–24‰). Ordinate—plasma chloride concentration (mM/l).

It has been suggested (Black, 1951) that ion uptake by soft tissues and bone may also reduce increases in extracellular electrolyte levels. Studies on the steelhead and brown trout (Gordon, 1959; Houston, 1959) indicate that this is insignificant as far as sodium and chloride are concerned. It may be of more importance with respect to divalent cations and certain anions. Calcium levels in the muscle and cellular fluids of steelhead trout, for example, rise sharply in sea water, although there is little change in the plasma concentration (Houston, 1959). However, it would seem that bone serves as a more important site of ion sequestration than muscle. The studies of Lovelace and Podoliak (1952) and Rosenthal (1956) have shown that bone will take up approximately ten times as much of an administered load of radio-calcium as muscle despite the disparity of their relative masses. These studies also indicate that the retention period of calcium in fish bone is extremely long. The basis for this may be inferred

from the work of Neuman and Weikel (1955) on mammalian bone. This indicates that excess extracellular phase calcium ions may enter unoccupied lattice positions ("faults") in bone crystals, and by overgrowth and intercrystalline exchange be removed from the bone crystal-interstitial fluid exchange surfaces. Since crystalline "faults" appear to be prevalent in the bone of juvenile and growing animals, this mode of sequestration may play a role of some importance in restricting the increase of calcium and other ion concentrations in the extracellular fluid during the adaptation process.

Finally, the work of Gordon (1963) on radio-chloride exchange in rainbow trout adapted to various salinities suggests the occurrence of adaptive decreases in the surface permeabilities of seawater-adapted animals, and in this supports conclusions reached earlier in studies on the stickleback (*Gasterosteus aculeatus*) (Mullins, 1951) and river lamprey (*Lampetra fluviatilis*) (Morris, 1960). Changes in membrane permeability may also be, in part at least, a consequence of the entry of seawater ions into the body fluid system. It is well established, for example, that calcium ions may markedly influence membrane permeability. Although the exact mechanism of this effect remains unestablished, Shanes (1960) has suggested that it may be attributable to the interaction of calcium ions with superficial membrane sites normally associated with univalent ion movement, and to an increase in the packing of membrane molecules at such sites. Since increases in the levels of cell-associated calcium have been observed in steelhead trout (Houston, 1959), and since such increases presumably extend to the branchial and integumentary epithelium as well as muscle, it would appear reasonable to speculate that calcium ion uptake may contribute to decreases in surface permeability, and thereby to the restriction of further ion uptake.

Decreases in pH have also been observed to cause decreases in membrane permeability, and it has been suggested by Shanes (1960) that the mechanisms involved may be similar to those associated with the calcium effect. While little work has been done on acid-base regulation in salmon during adaptation to sea water, Busnel (1943) observed that the pH of the blood of the rainbow trout decreased following transfer into sea water, an observation consistent with the decreases in plasma sodium:chloride ratio observed in several salmonid species during the adaptation process (Gordon, 1959; Houston, 1959; Parry, 1961). Thus, changes in extracellular fluid pH may also play some role in the causation of decreased surface permeability.

It would appear, then, that many of the changes induced in the body fluid system of the migratory salmonid following entry into sea water are, in a sense, passively self-restrictive. The uptake of sodium and chloride leads to the redistribution of tissue water with the result that incoming electrolyte is diluted at the cost of a relatively slight degree of cellular dehydration. Uptake of divalent cations by soft tissues and bone may restrict increases in extracellular electrolyte concentrations, and where associated with surfaces may result in decreases in permeability which further restrict ion uptake. Changes in pH may supplement or reinforce the latter action. Alone, passive effects of the types noted can only

reduce the rates and magnitudes of changes in the body fluid system, and will have no alleviative action. However, they may be of real importance in the adaptive process in the sense of providing time for the changes in regulatory activity which ultimately restore the internal state to the regulated condition.

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REFERENCES

BLACK, V. S. 1951. Osmotic regulation in fishes. *Univ. Toronto Stud. Biol. Ser.*, No. 59; *Publ. Ontario Fish Res. Lab.*, No. 71, pp. 53–89.

BUSNEL, R. G. 1943. Recherches de physiologie appliquées à la pisiculture: à propos de la migration de la truite arc-en-ciel. *Bull. Français Pisciculture*, No. 128, pp. 108–117.

GORDON, M. S. 1959. Ionic regulation in the brown trout (*Salmo trutta* L.). *J. Exper. Biol.*, **36**: 227–252.

1963. Chloride exchanges in rainbow trout (*Salmo gairdneri*) adapted to different salinities. *Biol. Bull.*, **124**: 45–54.

HOLMES, W. N., AND R. L. MCBEAN. 1963. Studies on the glomerular filtration rate of rainbow trout (*Salmo gairdneri*). *J. Exper. Biol.*, **40**: 335–341.

HOLMES, W. N., AND G. H. STOTT. 1960. Studies of the respiration rates of excretory tissues in the cutthroat trout (*Salmo clarki clarki*). II. Effect of transfer to sea water. *Physiol. Zool.*, **38**: 15–20.

HOUSTON, A. H. 1959. Osmoregulatory adaptation of steelhead trout (*Salmo gairdneri* Richardson) to sea water. *Canadian J. Zool.*, **37**: 729–748.

1960. Variations in the plasma-level of chloride in hatchery-reared yearling Atlantic salmon during parr-smolt transformation, and following transfer into sea-water. *Nature*, **185**: 632–633.

LOVELACE, F. E., AND H. A. PODOLIAK. 1952. Absorption of radioactive calcium by brook trout. *Progr. Fish Cult.*, **14**: 154–157.

MORRIS, R. 1960. General problems of osmoregulation with special reference to cyclostomes. *Symp. Zool. Soc. London*, No. 1, pp. 1–16.

MULLINS, L. J. 1951. Osmotic regulation in fish as studied with radioisotopes. *Acta Physiol. Scand.*, **21**: 303–314.

NEUMAN, W. F., AND J. H. WEIKEL. 1955. Recrystallization in bone mineral. *Ann. New York Acad. Sci.*, **60**(Art. 5): 685–695.

PARRY, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar* (L.) and some related species. *J. Exper. Biol.*, **37**: 425–434.

1961. Osmotic and ionic changes in blood and muscle of migrating salmonids. *J. Exper. Biol.*, **38**: 411–427.

ROSENTHAL, H. L. 1956. Uptake and turnover of calcium-45 by the guppy. *Science*, **124**: 571.

SHANES, A. M. 1960. Mechanism of change in permeability in living membranes. *Nature*, **188**: 1209–1210.

THREADGOLD, L. T., AND A. H. HOUSTON. 1964. An electron microscope study of the "chloride cell" of *Salmo salar* L. *Exper. Cell Res.*, **34**: 1–23.

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Effects of MS 222 on Glycogen and Lactate Levels in Rainbow Trout (*Salmo gairdneri*)

MS 222 (tricaine methanesulfonate, Sandoz) is employed widely as an anaesthetic for the handling and tagging of fishes. During investigations into the effects of injury on the glycolytic metabolism of rainbow trout, it became necessary to assess the effects of MS 222 anaesthesia. Published reports on the physiological results of anaesthesia led to conflicting inferences regarding possible biochemical changes. Randall (1962) observed a significant increase in heart rate, and variable increases in respiratory rate and amplitude in tench (*Tinca tinca* L.) at low doses of MS 222 (0.10–0.38 mM/liter). Larger doses (up to 0.77 mM/liter) frequently caused cardiac irregularity and respiratory collapse. McFarland (1959) reported an initial period of excitation in *Girella* and *Fundulus* at high concentrations of all anaesthetics tested. On the other hand, he found that the rate of oxygen consumption in *Fundulus* under light anaesthesia was significantly lower than that in control fish. He attributed the reduction to a lessening of the responses to initial handling and external stimuli, as well as to a significant lowering of the basal metabolic rate in anaesthetized fish. Bate-Smith and Bendall (1956) showed that rabbits killed by decapitation while immobilized with the skeletal muscle relaxant, mephenesin (quiet death), had levels of muscle glycogen 2½–3 times higher than those killed after stunning (violent death).

Fish used in this study were 1½-year-old rainbow trout (*Salmo gairdneri*), Oregon stock. Fish were transferred from an outside raceway to a covered indoor hatchery trough at least 3 days before use. They were fed 3 times a day on Clark's Grower Fish Feed calculated at 0.9% body weight per day. Water temperatures ranged from 10.8–11.0°C. Untreated control fish were dipped from the trough and sampled immediately. Experimental fish were dipped from the trough to a bucket of previously aerated water containing 0.5 mM MS 222 per liter (0.5 g/U.S. gal), and sampled after 60 sec exposure. No more than two fish were taken from one trough during any 4-hr working period. Method of obtaining blood by cardiac puncture from respiring fish, and method of estimating hemoglobin were those used by Black (1955). Muscle samples were taken from stunned fish, and were quick-frozen (Black *et al.*, 1962). Lactate was determined on trichloroacetic acid filtrates by the method of Barker and Summerson (1941). Glycogen was determined on KOH digests by the anthrone method of Carroll *et al.* (1956) after precipitation in 65% ethanol (Connor *et al.*, 1964).

Because of the rapidity of changes occurring in muscle glycogen and lactate levels during strenuous exercise in fish (Black *et al.*, 1962), the dosage of MS

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TABLE I. Total sampling times, body weights, and levels of hemoglobin, lactate, and glycogen in 1½-year-old rainbow trout (*Salmo gairdneri*) sampled without anaesthetic, and after 60 sec exposure to a 0.5-mM/liter solution of MS 222 (tricaine methanesulfonate, Sandoz). Results are presented as the mean ± the standard error of the mean. Water temperature 10.8–11.0°C (July 1963).

		Total sampling time		Body weight	Hemoglobin	Lactate		Muscle glycogen
		Blood	Muscle			Blood	Muscle	
		sec	*sec*	*g*	*g%*	*mg%*	*mg/100 g*	*% wet wt*
Untreated controls	Mean±SE	69±13.9	13±0.6	380.3±22.84	11.58±0.390	6.77±1.039	137.9±18.26	0.37±0.051
	No. of fish	10	10	20	10	10	10	10
	Range	30–163	10–16	220–634	9.35–13.80	4.65–15.46	73.7–266.9	0.18–0.62
Anaesthetized with MS 222	Mean±SE	31±5.0	11±0.7	413.9±16.40	11.08±0.287	5.65±0.501	97.7±12.66	0.29±0.033
	No. of fish	10	10	20	10	10	10	10
	Range	14–65	8–14	312–544	9.52–12.36	3.86–8.60	34.7–169.8	0.18–0.52

222 selected was high enough to produce complete loss of equilibrium and muscle tone within an average of 52±2.8 sec. This level of anaesthesia is equivalent to Stage II, Plane 2, or to Stage III as described by McFarland (1959 and 1960). The dosage was approximately twice the lethal dose recorded for *Fundulus* and might have proven fatal to these trout on prolonged exposure. However, when removed to a trough of fresh water after reaching the required level of anaesthesia, fish showed the first signs of recovery within an average of 57 sec, and were swimming upright, although sluggishly, within 166±10.4 sec. In this concentration of MS 222 the trout showed signs of initial stimulation during the first 15 sec as evidenced by agitated swimming. Failure of response to visual stimulation (Stage I, Plane 2) occurred at 21±1.2 sec, and the first signs of loss of equilibrium (Stage II, Plane 1) were observed at 26±1.5 sec.

Average sampling times and body weights, and average levels of hemoglobin, blood and muscle lactate and muscle glycogen for untreated and for anaesthetized fish are given in Table I. None of the differences in hemoglobin, lactate, or glycogen levels was statistically significant at the 5% level, although the muscle lactate level in fish treated with MS 222 was nearly one-third lower than that in fish sampled in the usual manner. Total sampling times for both muscle and blood were significantly reduced ($0.025 > P > 0.010$) by the use of MS 222. The faster sampling times were due mainly to a reduction in the time required to capture the fish and position them for sampling.

In conclusion, 60 sec exposure to a 0.5 mM/liter solution of MS 222 resulted in rapid and complete induction of a reversible Stage II or Stage III anaesthesia in 1½-year-old rainbow trout. Despite an apparent initial stimulation of muscular activity upon contact with the anaesthetic solution, levels of hemoglobin, glycogen, and lactate did not differ significantly from those in unanaesthetized fish. Slightly lower levels of lactate in muscle and blood of treated fish may have been related to faster sampling times and absence of struggling during sampling. It appears that use of this concentration of MS 222 for sampling during experiments on the glycolytic metabolism of rainbow trout does not effect the results adversely, and might even be advantageous to inexperienced workers especially under difficult field conditions.

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REFERENCES

BARKER, S. B., AND WILLIAM H. SUMMERSON. 1941. The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.*, **138**(2): 535–554.

BATE-SMITH, E. C., AND J. R. BENDALL. 1956. Changes in muscle after death. *Brit. Med. Bull.*, **12**: 230–235.

BLACK, EDGAR C. 1955. Blood levels of hemoglobin and lactic acid in some freshwater fishes following exercise. *J. Fish. Res. Bd. Canada*, **12**(6): 917–929.

BLACK, EDGAR C., ANNE ROBERTSON CONNOR, KWOK-CHEUNG LAM AND WING-GAY CHIU. 1962. Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. *J. Fish. Res. Bd. Canada*, **19**(3): 409–436.

CARROLL, NICHOLAS V., ROBERT W. LONGLEY AND JOSEPH H. ROE. 1956. The determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.*, **220**: 583–593.

CONNOR, ANNE R., CARL H. ELLING, EDGAR C. BLACK, GERALD B. COLLINS, JOSEPH R. GAULEY AND EDWARD TREVOR-SMITH. 1964. Changes in glycogen and lactate levels in migrating salmonid fishes ascending experimental "endless" fishways. *J. Fish. Res. Bd. Canada*, **21**(2): 255–290.

MCFARLAND, WILLIAM N. 1959. A study of the effects of anesthetics on the behaviour and physiology of fishes. *Inst. Marine Sci.*, **6**: 23–55.

1960. The use of anesthetics for the handling and the transport of fishes. *Calif. Fish and Game*, **46**(4): 407–431.

RANDALL, D. J. 1962. Effect of an anesthetic on the heart and respiration of teleost fish. *Nature*, **195**(4840): 506.

The Application of Gas Chromatography to the Identification of the Sterols of Scallop[1]

Numerous attempts in this Laboratory to isolate pure sterols, other than 24-methylenecholesterol, from molluscs indicated the need for a separation procedure based on properties other than solubility and chromatographic mobility. In the present investigation gas chromatography, which separates the sterols on the basis of carbon number, was used in conjunction with the more established procedures to study the total crude sterols of the adductor muscle of the scallop, *Placopecten magellanicus* (Gmelin).

The scallop sterols were separated from the total unsaponifiable fraction of the muscle fats by precipitation with digitonin and recovery of the sterols from the digitonides by treatment with pyridine (Bergmann, 1940).

The reaction of the crude sterol preparation with the Liebermann-Burchard reagent (Idler and Baumann, 1953) indicated the predominance of Δ^5-sterols in the mixture. The ultraviolet spectrum of the sterols in alcohol showed the presence of 0.8% $\Delta^{5,7}$-sterols (calculated as 7-dehydrocholesterol). The saturated sterols, isolated from a sample of the total sterol preparation according to the method of Schönheimer (1930), accounted for 4% by weight of the total.

For the gas chromatographic examination of the composition of the scallop sterols, the saturated sterols and methyl ether derivatives (Clayton, 1962) of the total native sterols were prepared. Also, the azoyl esters were prepared and separated into four zones by column chromatography and then hydrolyzed to give four free sterols fractions, α, β, γ, δ zones (Idler *et al.*, 1953). Analysis of each zone permitted the selection of an enriched fraction for the isolation of individual sterol components.

The apparatus used for gas–liquid chromatography was a Barber-Colman Model 10 with an argon ionization detector and glass columns (4 mm ID, 6 ft in length). For the analysis of the naturally occurring saturated sterols, the total sterols and their hydrogenated derivatives, the column was packed with 5% SE-30 on Anakrom ABS 70/80 mesh. The argon pressure was 30 psi and the temperatures (°C) were: column, 230; flash heater, 310; and detector cell, 275. The column for gas chromatography of the sterol methyl ethers was packed with 5% diethylene glycol succinate on Chromosorb W 60/80 mesh. The argon pressure was 15 psi and the temperatures (°C) were: column, 198; flash heater, 330; and detector cell, 212. Peak areas on the Barber-Colman were measured by a Disc Co. integrator and assumed to be equal to weight per cent. Column retention times (r_e) of peak areas were calculated relative to cholestane ($r_e = 1.0$). Column retention times were determined for a number of authentic sterol compounds to aid in the identification of unknowns.

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The naturally occurring saturated sterols were found by gas chromatography to consist of 59% C_{27}-sterols, 26% C_{28}-sterols, and 15% C_{29}-sterols. The chromatographic behaviour of the C_{27}-sterol (r_c = 2.05) indicated that this component was cholestanol rather than the isomeric 20-isocholestanol (r_c = 1.8).

Gas–liquid chromatography on SE-30 of the total scallop sterols showed 8 peak areas; the saturated derivatives of the total sterols gave 5 peak areas. Chromatography of the sterol methyl ethers on diethylene succinate gave 10 peak areas. All peak areas were designated by a letter and tabulated according to column retention time. The results are listed in Table I. On the basis of carbon number, the composition of the total sterol preparation was as follows: C_{27}-sterols, 42.4%; C_{28}-sterols, 41.9%; C_{29}-sterols, 9.6%; with small amounts of C_{26} and C_{30} compounds.

TABLE I. Composition and column retention times (r_c) of scallop sterols and derivatives as determined by gas chromatography.

	Native sterols							
Peak No.	X_1	A	B	C	D	X_2	E	F
r_c	1.26	1.83	2.02	2.30	2.66	3.2	3.5	4.3
Wt. %	6.4	14.1	26.8	16.3	26.0	2.2	8.2	<1

	Hydrogenated sterols				
Peak No.	X^1_1	$A^1 + B^1$	$C^1 + D^1$	$X^1_2 + E^1$	F^1
r_c	1.51	2.05	2.7	3.5	4.3
Wt. %	6.1	42.4	41.9	9.6	<1
Carbon No.		C_{27}	C_{28}	C_{29}	

	Sterol methyl ethers									
Peak No.	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}
r_c	2.3	3.2	3.66	4.4	4.8	5.6	6.2	7.1	7.4	8.2
Wt. %	5.4	2.6	34.3	17.2	4.6	23.2	6.3	1.0	4.5	2.2

The C_{27}-sterols contributed two peak areas, A and B. Peak A represented a single sterol, 22-dehydrocholesterol, a component isolated through the azoyl ester by Tamura *et al.* (1964). Peak B consisted of 2 sterols; cholestanol was the minor component, and cholesterol, the major one. Gas chromatography of the methyl ethers showed the presence of cholestanol, M_2, within Peak B. Attempts to identify cholesterol by isolation were not completely successful; further chromatography of the δ-zone sterols provided a fraction of over 90% purity. The main component of the purified fraction was shown to be a sterol with a saturated C_8 side chain by gas chromatographic analysis of the volatile products

of pyrolysis according to the method of Cox *et al.* (1958). The Liebermann-Burchard reaction (Idler and Baumann, 1953) and the infrared spectrum of the sterol established the presence of a double bond at the 5-position. The retention times of the saturated and methyl ether derivatives (M_3) also indicated that cholesterol was a component of the scallop sterols.

Peaks C and D represented the C_{28}-sterols. Peak C was identified as brassicasterol or its C_{24}-stereoisomer. A fraction from the δ-zone sterols, containing 74% peak C, was further purified by column chromatography and checked for purity by gas chromatography. Infrared spectrum analysis and the Liebermann-Burchard reaction showed this fraction to be a $C_{28}\Delta^{5,22}$-sterol. The melting points of the isolated fraction and derivatives were determined for comparison with values reported by Bergmann (1952) for authentic brassicasterol and corresponding derivatives. The results (published values in brackets) were as follows: free sterol, 151–152°C (148); sterol acetate, 153–154°C (157–159); sterol acetate dibromide, 219–222°C (236–238); sterol acetate tetrabromide, 193–196°C (209). The column retention time of Peak C equalled that of brassicasterol. The configuration of the methyl group at C_{24} would not be shown by gas chromatography under the conditions used here (Tsuda *et al.*, 1961) and the physical properties of the sterol and its derivatives suggest that some C_{24}-epimer may be present. Peak Area D was composed of two sterols, the main component (86%) being 24-methylenecholesterol. Gas chromatographic retention times of the saturated and methyl ether derivatives (M_6) were identical to those of 24-methylenecholesterol previously isolated from scallop by Fagerlund and Idler (1956). The minor component of Peak D was probably Δ^5-ergosterol; the retention time of the methyl ether derivative (M_5) equalled the retention time of authentic Δ^5-ergosterol methyl ether.

Peaks X_2 and E represented the C_{29}-sterols, none of which were positively identified. On the basis of its gas chromatographic retention time, Peak X_2 was tentatively identified as stigmasterol. Peak E appeared to be a mixture of C_{29}-unsaturated sterols. Gas chromatography of the methyl ether derivatives indicated a mixture of 50% β-sitosterol (M_7), 50% of an unidentified compound (M_9), and less than 1% fucosterol (M_8).

Gas chromatography showed the presence of two other groups of sterols, Peaks X_1 and F, which were not identified. The hydrogenation product of X_1 differed chromatographically from the original sterol but did not behave as a saturated C_{27}-sterol; its relative retention time indicated a lesser carbon number. The column retention time of Peak F was greater than for C_{29}-sterols and the chromatographic behaviour did not change after hydrogenation.

Although the present study does not fully describe the nature of scallop sterols, five components (cholestanol, cholesterol, 22-dehydrocholesterol, 24-methylenecholesterol, brassicasterol) which account for 80% of the total have been identified and measured quantitatively.

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REFERENCES

Bergmann, W. 1940. The splitting of digitonides. *J. Biol. Chem.*, **132**: 471–472.

1952. Sterols, Vol. 1, pp. 18–69. *In* Progress in the chemistry of fats and other lipids, Academic Press, Inc., New York.

Clayton, R. B. 1962. Gas-liquid chromatography of sterol methyl ethers and some correlations between molecular structure and retention data. *Biochemistry*, **1**: 357–366.

Cox, J. S. G., L. B. High and E. R. H. Jones. 1958. Determination of steroid side-chains. *Proceedings Chemical Society*, pp. 234–235.

Fagerlund, U. H. M., and D. R. Idler. 1958. Marine sterols. II. 24-Methylenecholesterol in molluscs. *J. Org. Chem.*, **21**: 372–373.

Idler, D. R., and C. A. Baumann. 1953. Skin sterols. III. Sterol structure and the Liebermann-Burchard reaction. *J. Biol. Chem.*, **203**: 389–396.

Idler, D. R., S. W. Nicksic, D. R. Johnson, V. W. Meloche, H. A. Shuette and C. A. Baumann. 1953. Separation and tentative identification of two new sterols from oats. *J. Am. Chem. Soc.*, **75**: 1712–1715.

Schönheimer, R. 1930. Method for the quantitative separation of unsaturated and saturated sterols. *Z. Physiol. Chem.*, **192**: 77–86.

Tamura, T., T. Wainai, B. Truscott and D. R. Idler. 1964. Isolation of 22-dehydrocholesterol from scallop. *Canadian J. Biochem.*, **42**: 1331–1337.

Tsuda, K., K. Sakai and N. Ikegawa. 1961. Steroid studies. XXX. Gas chromatography of C_{27}, C_{28}, and C_{29} sterols. *Chem. Pharm. Bull. (Tokyo)*, **9**: 835–836.

A Northern Pike, *Esox lucius*, with an Accessory Fin

In a study of the grass pickerel, *Esox americanus vermiculatus*, Crossman (1961) found two individuals with supernumerary fins on the dorsal surface. In the course of examining several thousand northern pike, *Esox lucius*, from Heming Lake, Manitoba, a single specimen was found in 1963 with an accessory fin on the ventral surface of the head.

The fish (28 cm fork length) had a small rayed-fin attached to one of the right anterior branchiostegal rays, on a line vertically drawn through the middle of the eye (Fig. 1A). In general outline, the fin resembled a pelvic fin. The ray count (approximately 9) was closer to the average number of pelvic rays for pike than pectoral rays. The fin was flexible, as though articulated. X-rays did not reveal any obvious bony support, but microscopic examination revealed a boomerang-shaped band to which the fin appeared to be attached.

The right side of the lower jaw, including the branchiostegals, was excised; the tissue was cleared and the bony parts stained (Fig. 1B). The staining revealed a definite malformation of the branchiostegal rays. From the posterior part of the lower jaw to the anterior part, the last three (3) branchiostegals appeared normal; the next, the fifth (5th), somewhat shorter than normal; the next, the fourth (4th), was boomerang-shaped and served to anchor the supernumerary fin. Above and slightly to the right of this curved bone is a bony stump which probably represents the base of the 4th branchiostegal; the next stump, probably representing the remains of the third (3rd) branchiostegal, was followed by a thick, relatively short bony structure which might have resulted from the fusion of the first (1st) and second (2nd) posterior branchiostegals; the anterior six (6) branchiostegals, not visible in the photograph, were normal. The branchiostegal count of 15 on the left side, which represents the average number for the species, was only one more than on the right if the boomerang-shaped bone and the proximal bony stump together represent only one branchiostegal. Occasionally, for this species a difference of one branchiostegal occurs between the two sides.

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REFERENCES

CROSSMAN, E. J. 1961. Grass pickerel, *Esox americanus vermiculatus*, with supernumary fins. *Copeia*, No. 2, pp. 235–236.

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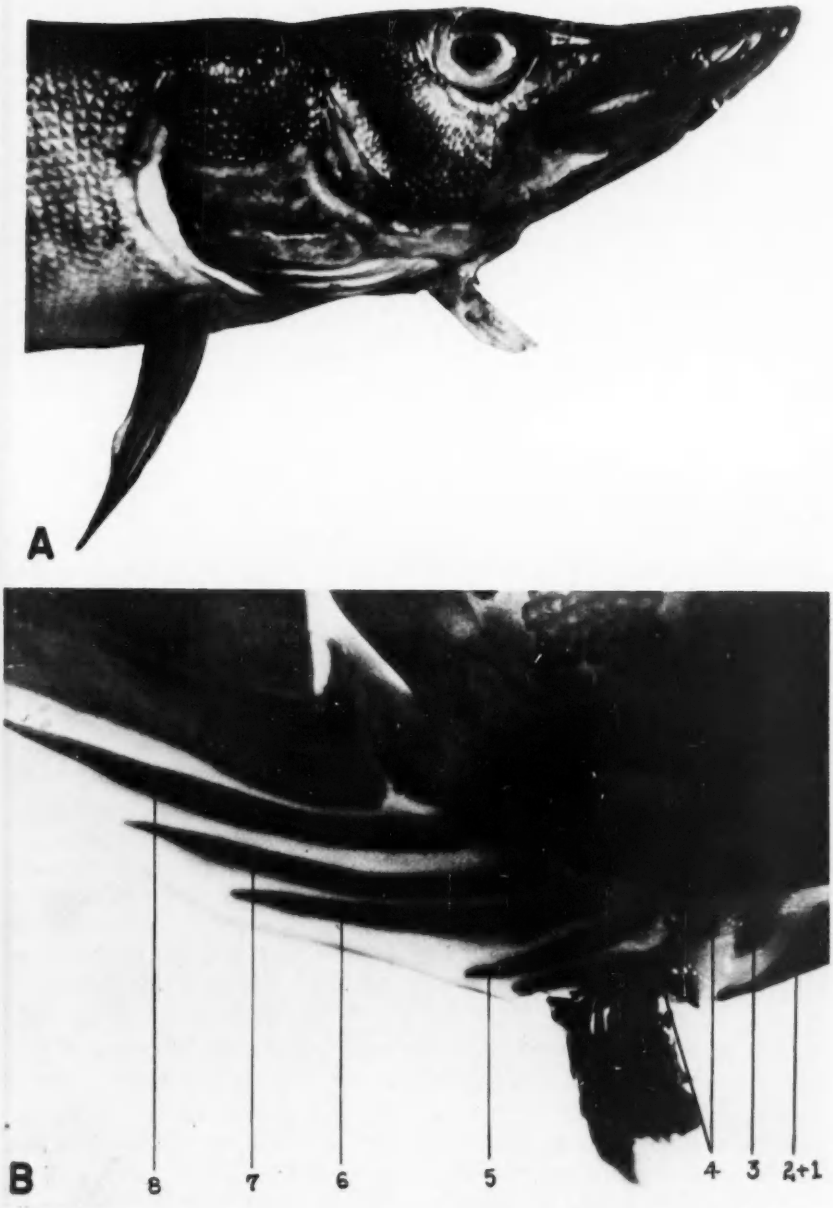

FIG. 1. A. Right side of pike (*Esox lucius*) head showing supernumerary fin; B. Cleared and stained section showing supernumerary fin attached to a malformed branchiostegal ray.

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Lobster (*Homarus americanus*) Tolerance for Tris Buffer, Sodium Fluoride, and Sea Water Extracts of Various Woods

Live lobsters are often held after capture in containers made from different woods and are sometimes maintained in artificial sea water, especially in inland areas. Wilder (1953), among others, has shown that lobsters can be held successfully in at least one artificial sea water formulation. However, in those municipalities which permit it, fresh water supplies are often fluoridated. Thus the artificial sea water made from these fresh water supplies also contains fluoride. Fluoride is toxic to one class of arthropods, the Insecta (Merck Index, 1960), and it is quite possible that it is also toxic to members of another class, the Crustacea. Consequently it is important to discover if fluoride is detrimental to live lobsters. Similarly, information on the toxicity to lobsters of different woods is required; in at least one instance high mortalities were suspected as being due to the use of western red cedar in lobster tank construction.

This study was designed to determine the toxicity to lobsters of woods commonly and infrequently used for tank construction and of sea water containing fluoride.

Tris buffer (tris(hydroxymethyl)aminomethane) was also tested for toxicity to lobsters. McFarland and Norris (1958) found that the debilitating acid conditions brought about by the accumulation of metabolic wastes could be controlled by tris buffer during the transport of fish in fresh sea water. Thus if this buffer was non-toxic to lobsters it might be usefully employed in certain experimental situations to regulate the acidity of natural and artificial sea water used for holding lobsters.

Sawdusts were made from clean, kiln-dried boards of redwood, black spruce, Douglas fir, eastern fir, western red cedar, and Tennessee cedar. Extracts were prepared from these sawdusts by shaking on a gyratory shaker 400 g of sawdust (dry wt) with 4 litres of clean filtered sea water at 24°C. At the end of 24 hr the supernatant fluid was removed by low vacuum filtration and the entire procedure was repeated twice more using 2 litres of clean sea water for each extraction. The fluid from the three extractions was pooled, filtered through Whatman No. 1 filter paper and used in the toxicity tests.

The sea water extracts of the various woods were added to clean, filtered sea water to give final extract concentrations of 25%.

Sodium fluoride (Baker Analyzed Reagent, J. T. Baker Chemical Co., Phillipsburg, N. J.) was added to clean filtered sea water to give final fluoride concentrations of 0.9, 2.25, and 4.5 ppm. Fluoride concentrations range from 0.7 to 1.0 ppm in artificially fluoridated water supplies (Merck Index, 1960).

Sufficient tris buffer (THAM, Fisher Scientific Co., Montreal, Que.) was added to clean, filtered sea water to give final concentrations of 2.2 and 4.4

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g/litre. The solution was adjusted to pH 7.8 (the value for local sea water) by the addition of concentrated HCl.

Live, vigorous lobsters, each weighing approximately 500 g, were exposed to the above materials for a period of 10 days in polyethylene tanks placed in a darkened refrigerated room maintained at 2°C throughout the test. The total volume of fluid in each tank was 15 litres. Air was constantly injected into the water but the water was not filtered or changed during the test.

Duplicate tanks were set up for each material or different concentration of the material and four lobsters placed in every tank. An exception was the control, which was run in quadruplicate. Thus each material or its different concentrations was tested against eight lobsters and compared with the 16 lobster controls. The results are shown in Table I.

TABLE I. Time to death (hr) for lobsters exposed to various materials. Duration of test 240 hr; temperature 2°C

	Lobster number							
Materials tested	1	2	3	4	5	6	7	8
Tris 2.2 and 4.4 g/litre	–	–	–	–	–	–	–	–
Redwood	–	–	–	–	–	–	–	–
Black spruce	–	–	–	–	–	–	–	–
Douglas and eastern fir	–	–	–	–	–	–	–	–
Western red cedar	56	56	56	56	80	128	80[a]	80[a]
Tennessee cedar	–	–	–	–	–	–	–	–
Fluoride 0.9, 2.25, and 4.5 ppm	–	–	–	–	–	–	–	–
Controls 1 and 2	–	–	–	–	–	–	–	–

[a]Extremely weak from 80 hr to conclusion of test.

The results show conclusively that western red cedar contains a sea water extractable material which is toxic to lobsters and therefore this wood should not be used for lobster holding tanks. The toxic material could well belong to the class of polyoxyphenols extracted from western red cedar by Gardner *et al.* (1959) and characterized by Gardner *et al.* (1960). Western red cedar should be added to the list of materials shown to be toxic to lobsters by Wilder (1952).

The redwood and Tennessee cedar extracts did not harm the lobsters but were highly coloured. The redwood extract heavily stained the lobsters' shells. This stain persisted through the cooking process and would militate against the use of this wood for holding lobsters. The other woods, black spruce, Douglas fir, and eastern fir, were non-toxic and thus would appear from this point of view to be suitable for lobster tank construction.

Fluoride was not toxic even at levels five times those generally used in municipal water supplies. Thus it appears that fluoridated water can be used safely for the preparation of artificial sea water for the holding of live lobsters.

Tris buffer was non-toxic. Since the temperature was low the lobsters' metabolic rate was also low and the increase in acidity was minimal. The pH value for the tris buffered tanks dropped no more than 0.4 pH units and the sea water in the other tanks dropped at least 0.8 unit. It is apparent that tris buffer concentrations up to the levels tested could be used for regulating acidity in lobster tanks.

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REFERENCES

GARDNER, J. A. F., G. M. BARTON AND H. MACLEAN. 1959. The polyoxphenols of western red cedar (*Thuja plicata* Donn.). I. Isolation and preliminary characterization of plicatic acid. *Canadian J. Chem.*, **37**: 1703–1709.

GARDNER, J. A. F., B. F. MACDONALD AND H. MACLEAN. 1960. The polyoxphenols of western red cedar (*Thuja plicata* Donn.). II. Degradation studies on plicatic acid, a possible lignan acid. *Canadian J. Chem.*, **38**: 2387–2394.

MCFARLAND, W. N., AND K. S. NORRIS. 1958. The control of pH by buffers in fish transport. *California Fish and Game*, **44**: 291–310.

MERCK INDEX. 1960. Merck and Co., Inc., Rahway, New Jersey, 7th ed.

WILDER, D. G. 1952. The relative toxicity of certain metals to lobsters. *J. Fish. Res. Bd. Canada*, **8**: 486–487.

1953. Holding live lobsters in aerated artificial sea water. *Fish. Res. Bd. Canada, St. Andrews Biol. Sta. Circular, General Series*, No. 21, 4 pp.

CORRECTIONS FOR RECENT PUBLICATIONS OF THE FISHERIES RESEARCH BOARD OF CANADA

Journal of the Fisheries Research Board of Canada

VOLUME 18, NO. 2—paper by E. Bilinski: "BIOSYNTHESIS OF TRIMETHYLAMMONIUM COMPOUNDS IN AQUATIC ANIMALS. II. ROLE OF BETAINE IN THE FORMATION OF TRIMETHYLAMINE OXIDE BY LOBSTER (*Homarus americanus*)."

Page 285: Table I, under "Administered to lobsters"—for "78,000" read "780,000".

VOLUME 18, NO. 4—paper by K. S. Ketchen: "OBSERVATIONS ON THE ECOLOGY OF THE PACIFIC COD (*Gadus macrocephalus*) IN CANADIAN WATERS."

Page 534: Para. 6, line 5, "duration" should read "deviation".

VOLUME 20, NO. 1—paper by J. R. McBride, U. H. M. Fagerlund, M. Smith and N. Tomlinson. "RESUMPTION OF FEEDING BY AND SURVIVAL OF ADULT SOCKEYE SALMON (*Oncorhynchus nerka*) FOLLOWING ADVANCED GONAD DEVELOPMENT."

Figure 4 facing page 98: Legend should read "Force-fed castrated male (*bottom*), unfed and unoperated male control (*top*) . . . "

VOLUME 20, NO. 2—paper by F. D. McCracken: "SEASONAL MOVEMENTS OF THE WINTER FLOUNDER, *Pseudopleuronectes americanus* (WALBAUM), ON THE ATLANTIC COAST."

Page 570: "Table IX.A." should read "Table XI.A."

VOLUME 20, NO. 4—paper by C. C. Lindsey and T. G. Northcote: "LIFE HISTORY OF REDSIDE SHINERS, *Richardsonius balteatus*, WITH PARTICULAR REFERENCE TO MOVEMENTS IN AND OUT OF SIXTEENMILE LAKE STREAMS."

Page 1016: In Fig. 10, the number "18,314" under "Lake Population" should read "13,151".

VOLUME 20, NO. 5—paper by A. T. Pinhorn and C. W. Andrews: "EFFECT OF PHOTOPERIODS ON THE REACTIONS OF JUVENILE ATLANTIC SALMON (*Salmo salar* L.) TO LIGHT STIMULI."

Page 1252: Para. 3, line 3, "4240–5200 hr (265–325 days)" should read "4720–5136 hr (295–321 days)".

Page 1257: Para. 6, line 3, "2176–4000 hr (136–250 days)" should read "3296–3936 hr (206–246 days)".

VOLUME 20, No. 6—paper by Robert R. Parker: "ON THE PROBLEM OF MAXIMUM YIELD FROM NORTH PACIFIC SOCKEYE SALMON STOCKS."

Page 1384: Para. 3, lines 10, 11, 13, and 14, "*g*" should read "*q*".

VOLUME 21, No. 3—paper by T. K. Pitt: "FECUNDITY OF THE AMERICAN PLAICE, *Hippoglossoides platessoides* (FABR.) FROM GRAND BANK AND NEWFOUNDLAND AREAS."

Page 606: Para. 5, line 2, "0.084" should read "0.804".

VOL. 21, No. 4—paper by S. J. Westrheim: "ROCKFISH (*Sebastodes brevispinis*) IN BRITISH COLUMBIA WATERS."

Page 855: Para. 4, line 6, "8–10" should read "9–10"; "44–49" should read "56–70"; line 7, "33–36" should read "28–31".

Bulletins of the Fisheries Research Board of Canada

BULLETIN No. 130, by S. N. Tibbo, L. R. Day and W. F. Doucet: "THE SWORDFISH (*Xiphias gladius* L.), ITS LIFE-HISTORY AND ECONOMIC IMPORTANCE IN THE NORTHWEST ATLANTIC."

Page 12: Last line, instead of "61°F" read "60°F".

BULLETIN No. 133, by P. F. Elson: "PREDATOR-PREY RELATIONSHIPS BETWEEN FISH-EATING BIRDS AND ATLANTIC SALMON."

Page 73: Appendix I, section A(b) should read

"(b) Eaten in 19 merganser-days—18.75 lb (White, 1937).

Eaten in 657 merganser-days—449.50 lb (White, 1957, Table IB).

$$\text{Average daily consumption } \frac{468.25}{676} = 0.693 \text{ lb.}$$

$$\text{Value used} = \frac{1.051 + 0.693}{2} = \text{about 1 lb."}$$

RECENT ARTICLES ON INVESTIGATIONS OF THE FISHERIES RESEARCH BOARD OF CANADA PUBLISHED IN OTHER PERIODICALS

Studies No.

873. **Taylor, F. H. C.** 1963. The stock-recruitment relationship in British Columbia herring populations. Rapp. Procès-Verbaux Réunions, Conseil Perm. Intern. Exploration Mer, **154**: 279–292.

874. **Templeman, Wilfred.** 1963. Otter-trawl covered codend and alternate haul mesh-selection experiments on redfish, haddock, cod, American plaice and witch flounder: girth measurements of haddock, cod and redfish and meshing of redfish in the Newfoundland area. ICNAF, Spec. Publ., No. 5, pp. 201–217.

875. **Templeman, Wilfred.** 1963. Comparison of returns from different tags and methods of attachment used in cod tagging in the Newfoundland area, 1954 and 1955. ICNAF, Spec. Publ., No. 4, pp. 272–287.

876. **Templeman, Wilfred, and T. K. Pitt.** 1963. Comparison of returns from different tags and tagging methods for cod tagged in the inshore area, St. John's and Fogo, Newfoundland, 1950. ICNAF, Spec. Publ., No. 4, pp. 294–299.

877. **Sandeman, E. J., and E. I. S. Rees.** 1963. Some notes on the tagging of redfish, *Sebastes marinus* (L.), from deep water. ICNAF, Spec. Publ., No. 4, pp. 300–305.

878. **Templeman, Wilfred, and A. M. Fleming.** 1963. Distribution of *Lernaeocera branchialis* (L.) on cod as an indicator of cod movements in the Newfoundland area. ICNAF, Spec. Publ., No. 4, pp. 318–322.

879. **Tamura, T., T. Wainai, B. Truscott and D. R. Idler.** 1964. Isolation of 22-dehydrocholesterol from scallop. Canadian J. Biochem., **42**(9): 1331–1337.

880. **Wylie, V., and M. Smith.** 1964. Nucleotides of the Pacific oyster *Crassostrea gigas* (Thunberg). Canadian J. Biochem., **42**(9): 1347–1351.

881. **Sprague, J. B., and R. L. Saunders.** 1963. Avoidance of sublethal mining pollution by Atlantic salmon. Proceedings of the 10th Ontario Industrial Waste Conference, Ontario Water Resources Commission, pp. 221–236.

882. **Lantz, A. W.** 1964. Fish cold storage plant; Mutwal, Ceylon. ASHRAE Journal (Am. Soc. Heating, Refrig. Air-cond. Eng. J.), pp. 38–40, August 1964.

883. **Ackman, R. G., and J. C. Sipos.** 1964. Application of specific response factors in the gas chromatographic analysis of methyl esters of fatty acids with flame ionization detectors. J. Am. Oil Chemists' Soc., **41**(5): 377–378.

884. **Vanstone, W. E., Eve Roberts and H. Tsuyuki.** 1964. Changes in the multiple hemoglobin patterns of some Pacific salmon, genus *Oncorhynchus*, during the parr-smolt transformation. Canadian J. Physiol. Pharm. (In press.)

885. **Johnson, W. E.** 1964. Quantitative aspects of the pelagic, entomostracan zooplankton of a multibasin lake system over a 6-year period. Verh. Internat. Verein. Limnol., **15**: 727–734.

Copies of individual reprints, if available, may be obtained from the authors or Board establishments through which the investigations were conducted.

Reprints of these articles are included in an annual volume of "Studies", composed of two parts. These are distributed to a selected mailing list. (Articles numbered 776 to 817 appeared in the Studies for 1963, Part 1.)

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5: 1183 (effect on swimming performance of young sockeye salmon)
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